HYPERPROLACTINEMIA AND MALE REPRODUCTIVE FUNCTIONS

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CHAPTER I

HYPERPROLACTINEMIA IN MAN

1. Introduction

The importance of prolactin (PRL) as a regulator of reproductive functions in mammals and birds was established within a few years of its discovery in 1928 (Stricker & Grueter, 1928). Since the isolation and specific radioimmunological determination of human PRL (Guyda et al., 1971; Hwang et al., 1971), PRL has become a hormone of considerable clinical interest especially in the diagnosis and management of pituitary tumors. However, up to now little is known about the physiologic role of PRL in man. The principal action of PRL in mammals in general is stimulation of milk formation in the hormonally prepared female breast (Ganong, 1980). Furthermore hyperprolactinemia, whether due to hypothalamic-pituitary disorders, drug use, hypothyroidism or other diseases, is associated with reproductive dysfunctions in men and women (see review von Werder et al., 1977; Kirby et al., 1979). The association of hyperprolactinemia with galactorrhea and menstrual irregularities has been recognized for many years (Jacobs et al., 1976).

Hyperprolactinemia in male patients does not cause "typical" manifestations and may not be recognized by the patient or his physician (Carter et al., 1978). Impotence, loss of libido, hypogonadism, impaired spermatogenesis, gynecomastia and galactorrhea can be observed (see review Hermanns & Hafez, 1981). Hyper-

prolactinemia can be detected in male patients with infertility, impotence and hypogonadism.

The mechanisms by which hyperprolactinemia lead to reproductive dysfunctions have not yet been established. Stimulatory as well as inhibitory effects of increased PRL levels on reproductive functions have been described, as will be apparent from the next paragraphs. Several hypotheses have been proposed to account for these dysfunctions in hyperprolactinemic males:

a. Interference by PRL at the hypothalamic and/or pituitary level.
b. Interference at the peripheral level:
   - Blockade of the effects of gonadotropins at the gonadal level.
   - Functional impairment of the accessory sex glands indicating a possible interaction between PRL and testosterone.
   - Direct impairment of sperm motility and/or other sperm qualities.
c. Alterations in adrenal steroidogenesis.

In order to elucidate the mechanisms involved in the relationship between PRL and reproductive functions, we have collected information on four groups of male patients:

1. Men with hyperprolactinemia caused by PRL-secreting pituitary adenomas (paragraph: 2).
2. Healthy volunteers in whom hyperprolactinemia was induced by neuroleptics and other drugs (paragraph: 3.1).
3. Men with other diseases associated with hyperprolactinemia (paragraph: 3.2).
4. Men with infertility and/or impotence (paragraph: 4).

Relevant information about these groups of patients as reported in the literature will be discussed in the next paragraphs.

2. PRL-secreting pituitary adenomas

In 1972 a case of a 24 yr-old male with idiopathic galactorrhea and mild hypogonadism has been reported (Volpé et al., 1972). This patient presented with gynecomastia and galactorrhea. Libido and penile erections were normal. He had mild hypogonadism, manifested by low plasma testosterone levels and oligozoospermia. A testicular biopsy showed histologically moderate gonadal atrophy. Basal serum gonadotropin levels were normal, while serum PRL levels were elevated. An explanation for the increased PRL levels could not be found; X-rays of the skull and pneumencephalography were normal. However, the findings in this patient could fit with the presence of a PRL-secreting microadenoma of the pituitary (tumor less than 1 cm in diameter, sella described as not enlarged).

Costello (1936) showed small pituitary adenomas in 225 of 1000 routine autopsies. In a recent study (Burrow et al., 1981), microadenomas were found in 32 of the 120 pituitaries removed at autopsy. Forty-one percent of these microadenomas stained for PRL. Twenty-seven of the 120 subjects had tomographic abnormalities compatible with the presence of a microadenoma. In 26 of 93 patients with a negative tomography, a microadenoma was found.

It is not surprising that prolactinomas are the most frequently diagnosed pituitary tumors both in men and women (Frantz, 1978). In contrast to the

many reports of prolactinomas in women, PRL-secreting adenomas in men have been much less extensively studied (Grisoli et al., 1980). Several of these reports on men with hyperprolactinemia and radiologically abnormal sellas are summarized in Table 1.1. In cases of a normal sella turcica the diagnosis prolactinoma was made by excluding other causes of hyperprolactinemia.

Differentiation between functional and tumorous hyperprolactinemia has to be achieved by several diagnostic procedures: repeated PRL determinations, dynamic tests, polytomography of the sella, computerized axial tomography (CAT), visual fields etc.

2.1. Symptoms and signs

In contrast to the findings in the above mentioned patient of Volpé, disturbances of potency and libido are prominent features in the majority of men with a PRL-secreting pituitary adenoma. Gynecomastia and galactorrhea have been less frequently described in men with prolactinomas (Table 1.1). Franks et al. (1976) suggested that in untreated patients with the combination of acromegaly and hyperprolactinemia impotence might be caused by PRL. Four of their 5 hyperprolactinemic men complained of loss of potency and libido. No improvement of these complaints was seen after operation in 2 of them, whose PRL levels were still high, while growth hormone (GH) levels normalized. Some patients recognized a decreasing volume of their seminal ejaculate (Thorner et al., 1977).

TABLE 1.1 Clinical features of untreated men with prolactinomas.

number of patients	diminution or loss of libido %	impotence %	gynecomastia %	galactorrhea %	author	
22	91	91	14	14	Carter et al.,	1978
8	75	100	50	38	Buvat et al.,	1978
21 8[a]	76 75	76 75	10	10	Franks et al.,	1978
10 15	n.m.	68	n.m.	28	Thorner & Besser,	1978
30	80	80	33	30	Derome et al.,	1979
25	n.m.	88	40	20	Peillon et al.,	1979
22	95	n.m.	27	18	Grisoli et al.,	1980
15	80	80	27	20	Serri et al.,	1980
57 24[b]	98	98	28	14	Eversmann et al.,	1981
8	n.m.	87	12	12	Prescott et al.,	1982
26	100	100	n.m.	n.m.	Spark et al.,	1982

n.m., not mentioned
[a] acromegaly
[b] after surgery

The testes may be of normal or small size and are usually soft. In some patients the prostate is small.

Surprisingly enough, relative few patients with impotence and loss of libido seek medical advice. The presenting clinical signs comprised headache, visual field defects or signs of hypopituitarism, caused by intra-, supra- and para-sellar extension of tumor mass. Retrospectively, sexual disturbances appeared to have existed for a long time.

2.2. Gonadotropins

In 1974 basal serum gonadotropins and their responses to luteinizing hormone-releasing hormone (LRH) in 4 hyperprolactinemic men were presented by Thorner et al.. Both basal and stimulated values of LH and FSH turned out to be normal. The results of other studies are listed in Table 1.2.
From these observations it can be learned that basal gonadotropin levels in untreated hyperprolactinemic men are mostly normal with a normal or decreased rise after administration of LRH.

Recently Eversmann et al. (1981) showed an impaired response of LH and FSH to LRH stimulation in 86% of 57 hyperprolactinemic men. In this study 49 patients had a macroadenoma of whom 24 underwent pituitary surgery before the start of the study. Normal responses of serum gonadotropins to LRH have also been reported in men with macroadenomas (Table 1.2). An exaggerated response of gonadotropins to LRH as described in hyperprolactinemic women and especially in women with microadenomas (Asfour, 1977; Thorner & Besser, 1978; Monroe et al., 1981), is not a common finding in men.

TABLE 1.2 Basal serum gonadotropins and their response to LRH in untreated hyperprolactinemic men.

number of patien+-	LH basal	Δ LH	FSH basal	Δ FSH	sellar region	author
1	n	n.m.	n	n.m.	normal	Volpé et al.
22	↓(7/15)	n(9/9)	↓(5/15) ↑(2/15)	n(9/9)	macroadenomas[a]	Carter et al.
1	n	n	n	n	macroadenoma	Hsu et al.
1	n	n.m.	n	n.m.	normal	McKenna et al.
25	n	↓	n	↓	macroadenomas	Peillon et al.
9	n	↓	n	n	n.m.	Snyder et al.
3	↓	↓(2/3) ↑(1/3)	↓	↓(2/3) ↑(1/3)	macroadenomas	Luboshitsky et al.
15	↓(3/15)	n(6/6)	↓(7/15)	n(6/6)	macroadenomas(14/15)	Serri et al.
22	↓(1/20)	↓(13/19)	n.m.	n.m.	macroadenomas	Grisoli et al.
57[b]	n.m.	↓(49/57)	n.m.	↓(49/57)	macroadenomas(49/57)	Eversmann et al.
8	n(8/8)	n(8/8)	n.m.	n.m.	macroadenomas(7/8)	Prescott et al.
1	n	↓	n	↓	microadenoma	Davis
16	↓	↓	n.m.	n.m.	macroadenomas	Spark et al., 1982
10	n	n	n.m.	n.m.	normal	
n(%)	98(78.4)	48(29.3)	47(66.2)	58(52.3)		total
↓(%)	27(21.6)	115(70.1)	22(30.9)	52(46.8)		
↑(%)	-	1(0.6)	2(2.9)	1(0.9)		

n=normal ↓=decreased ↑=increased ()=number of patients compared to total number investigated
n.m.=not mentioned; [a] majority of patients; [b] 24 after surgery

Boyar et al. (1974) and Bohnet et al. (1975) studied episodic LH secretion in hyperprolactinemic patients. Boyar et al. (1974), having studied 7 women and 2 men, found normal mean serum LH levels over 24 hours and normal episodic fluctuations of LH concentrations in those hyperprolactinemic patients with a normal sella turcica and presumably a microadenoma. LH concentrations were low and episodic LH peaks absent in patients with radiologically obvious pituitary tumors (macroadenomas). In the experience of Bohnet et al. (1975) episodic LH peaks were absent in 12 of 14 hyperprolactinemic women.

In many hyperprolactinemic male patients testosterone levels have been reported to be low. The observations of "normal" basal gonadotropin levels together with normal or even exaggerated responses of gonadotropins to LRH in patients with prolactinomas suggest that the ability of the pituitary to secrete LH and FSH is intact.

It has been postulated that PRL might inhibit endogenous LRH release, although in human studies changes in LRH secretion can only be inferred from the study of peripheral gonadotropin levels.

A relationship between low gonadotropin levels and elevated PRL secretion was originally proposed by Ben David et al. (1971) in rats. Hyperprolactinemia in rats resulted in an increased hypothalamic dopamine turnover rate, associated with decreased LH secretion (Hökfelt & Fuxe, 1972). If extrapolation of these data to men is justified, dopamine might also be involved in gonadotropin secretion in hyperprolactinemic patients. In normal men and women dopamine-induced inhibition of LH secretion could be demonstrated, as shown by a reduction in both pulse frequency and amplitude of LH secretion (Leblanc et al., 1976;

Huseman et al., 1980; Ropert et al., 1981). Lachelin et al. (1977) reported a significant fall in LH and FSH levels following the administration of a single dose of bromocriptine (2.5 mg orally) to 6 women with hyperprolactinemia. However, according to Thorner et al. (1980b) the same dose of bromocriptine given to 12 hyperprolactinemic women resulted in suppression of PRL levels, without any effect on LH secretion. The latter finding shows that bromocriptine obviously had no effect on serum gonadotropins.

There are experimental data in healthy volunteers of both sexes which suggest that opioids might be involved in regulating LH release (Grossman et al., 1981). Recently, the suppression of serum LH levels in women with PRL-secreting microadenomas have been explained by an increase of endogenous opioids (Lightman et al., 1980; Quigley et al., 1980; Lamberts et al., 1981; Lightman et al., 1981). Intravenous infusion or bolus injections of naloxone resulted in a rise of plasma LH in these hyperprolactinemic women.

It remains difficult to find a relationship between PRL and serum gonadotropin levels in hyperprolactinemic men, since most of the data are related to the presence of macroadenomas. In those situations destruction or local compression of normal pituitary tissue can be postulated. In hyperprolactinemic women, however, there are some indications that elevated PRL levels can disturb serum gonadotropins by alterations of hypothalamic dopamine release, by suppression of the pulsatile LRH secretion, and by an increase in endogenous opioids.

2.3. Testicular functions

2.3.1. Testosterone production

From data on blood testosterone concentration under physiological and experimental conditions it appears that PRL may stimulate testicular steroidogenesis in men. The sleep related increase of plasma testosterone in adult men is partially due to increased PRL levels (Rubin et al., 1975, 1976). Men with prolactinomas have low concentrations of plasma testosterone (Eversmann et al., 1981), which may in some patients be secondary to local destruction and/or compression of normal pituitary tissue, caused by a macroadenoma and resulting in LH deficiency. Nevertheless, low testosterone levels have recently also been reported in men with microadenomas (Spark et al., 1982).

Changes in plasma testosterone levels after human chorionic gonadotropin (hCG) administration to hyperprolactinemic men have been found to be normal (Carter et al., 1978; Turpin et al., 1979b; Luboshitzky et al., 1979), but not in others (Fonzo et al., 1977; Thorner et al., 1977; Peillon et al., 1979). The cases, where blood levels of LH and FSH in hyperprolactinemic men are normal, the elevated PRL levels may interfere with the action of gonadotropins on the gonads (Besser & Thorner, 1975). Further, hyperprolactinemic hypogonadism could be due to the interference of increased PRL with conversion of testosterone into biologically active dihydrotestosterone (DHT) which mainly occurs in peripheral tissues (Ito & Horton, 1971). Data in support of this view have been published by Lackritz & Bartke (1980). However, Turpin et al. (1979b) were not able to show such an ab-

normality in hyperprolactinemic men.

2.3.2. Spermatogenesis

Contradictory results have been reported with respect to the literature of PRL on spermatogenesis. Elevated PRL levels have been found in patients with azoospermia (Suominen et al., 1979), oligozoospermia (Volpé et al., 1972; Luboshitzky et al., 1979), normozoospermia (Carter et al, 1978) and even polyzoospermia (Peillon et al., 1979; Snyder et al., 1979). Infertility was present in 10 out of a group of 25 patients with prolactinomas (Peillon et al., 1979).

Testicular biopsies may show testicular atrophy (Volpé et al., 1972) or normal spermatogenesis (Jequier et al., 1979). Infertility, however, may not only be due to hyperprolactinemia but also to hypogonadism or impotence accompanying hyperprolactinemia. An example of such a possibility was published by Franks et al. (1978), who reported a hyperprolactinemic patient with hypogonadism and infertility, who was treated with hCG. Although the sperm counts were less than one million per ml, he fathered a child. Another interesting observation on a man with a PRL-secreting pituitary tumor was made by Baccetti et al. (1979), who reported bicellular spermatozoa, which disappeared after correction of the PRL levels, suggesting an effect of PRL on spermatogenesis. The patient had 3 children, the youngest was seven years old at the time of diagnosis. After PRL level reducing therapy another son was born. Another patient with infertility and impotence was described by Hsu et al. (1978). Previously he underwent transsphenoidal adenomectomy of a PRL-secreting tumor, after

which PRL levels remained elevated. Sperm examination revealed azoospermia. Treatment with LH and FSH improved sperm parameters and his wife conceived 6 months after the start of treatment.

This review on the literature considering spermatogenesis in men with prolactinomas reveals that sometimes sperm abnormalities, both quantitative and qualitative, are present. It remains unclear whether the sperm abnormalities or infertility may be explained by the elevated PRL levels or by suppression of LH, FSH and testosterone.

2.4. Adrenals

Following the recognition in some female patients of the association of hyperprolactinemia with mild hirsutism, there has been a search for abnormal adrenal steroid production. To study effects of PRL on adrenocortical function, dehydroepiandrosterone (DHA), dehydroepiandrosterone sulphate (DHAS), androstenedione and testosterone were measured in serum obtained from hyperprolactinemic women (Carter et al., 1977). DHAS in hyperprolactinemic women was slightly higher than in controls ($p<0.02$). Vermeulen et al. (1977) described increased DHA and DHAS levels in young women with prolactinomas. High levels for DHA were also found in 3 post-menopausal hyperprolactinemic women, and for DHAS in two of them. In 7 males with a PRL-secreting pituitary tumor only DHAS levels were significantly increased compared to normal controls (Vermeulen & Ando, 1978). Similar observations were presented by others (Giusti et al., 1978; Kandeel et al., 1978; Jones et al., 1980; Lobo et al., 1980; Seki & Kato, 1981). Other inves-

tigators have not found elevated levels of DHA and DHAS (Parker et al., 1977; Thorner & Besser, 1978; Metcalf et al., 1979; Turpin et al., 1979a; Belisle & Menard, 1980; Drucker & David, 1980; Facchinetti et al., 1980). Administration of ovine PRL to 3 normal men did not increase DHA, DHAS and testosterone concentrations (Varma et al., 1977).

3. Hyperprolactinemia not related to pituitary tumors

It is now well established that hyperprolactinemia is caused by or associated with a variety of pathologic states such as hypothalamic disorders, Cushing's disease, acromegaly, hypothyroidism, renal diseases, drugs etc. (Besser & Thorner, 1976; Lamberts et al., 1978; Frantz, 1978; del Pozo & Brownell, 1979).

3.1. Drug-induced hyperprolactinemia

Several drugs have been used to induce hyperprolactinemia and to investigate its effects on reproductive functions.

Suppressed testosterone levels were found in men in whom hyperprolactinemia was induced by metoclopramide (Carter & Friesen, 1976). In another study, Magrini et al. (1979) reported a decreased conversion of testosterone into DHT after injection of a long-acting testosterone preparation during metoclopramide-induced hyperprolactinemia.

Metoclopramide had no effect on either basal levels of LH, FSH and testosterone or the response of LH and FSH to LRH (Falaschi et al., 1978).

Treatment with metoclopramide did not reveal any

effect on spermatograms (Dorow et al., 1981), although a short term increase of total sperm count was seen 1 week following initiation of metoclopramide treatment (Jecht et al., 1980).

When hyperprolactinemia was induced by sulpiride in normal volunteers, the increase of serum testosterone levels in response to hCG was significantly higher (Ambrosi et al., 1976). The increase of DHT levels in response to hCG appeared to be diminished (Magrini et al., 1976). Recently it was shown that the increase of both testosterone and DHT in response to hCG was significantly higher in men with sulpiride-induced hyperprolactinemia (Martikainen & Vihko, 1982).

Haloperidol-induced hyperprolactinemia was able to raise basal testosterone levels (Rubin et al., 1976; 1978). Infusion of haloperidol was able to suppress the LRH-induced release of LH (del Carmen Diaz et al., 1981).

It can be concluded from these studies that drug-induced hyperprolactinemia in healthy men may lead to increased basal or hCG-stimulated testosterone levels. Some observations also suggest a suppression of 5α-reductase activity in hyperprolactinemic states. Further, a direct effect of the drugs on hypothalamic, pituitary and testicular functions may also be possible.

3.2. Hyperprolactinemia in renal disease

The kidney is the main site of PRL elimination (Bauer et al., 1980). In 16 out of 21 consecutive adult male patients with chronic renal failure PRL levels were elevated (Hagen et al., 1976). Impotence

was found in 11 of these 21 patients but plasma PRL levels were elevated in only 6 of the 11 impotent men. Three patients had gynecomastia and elevated PRL levels, none had galactorrhea. LH was decreased in 3 patients, FSH wat not decreased in any of the patients but was elevated in 15 of 21 patients. In other series hyperprolactinemia not attributable to drugs was found in 60 out of 349 patients of both sexes with renal disease, comprising 210 patients with impaired renal functions, 87 with moderate chronic renal failure and 52 with severe chronic renal failure (Cowden et al., 1978), in 14 of 18 patients with chronic renal failure (Lim et al., 1979) and in 7 of 33 men with end-stage renal failure (Gomez et al., 1980). In the last series information on libido and potency was obtained from 22 out of 33 men investigated. Elevated PRL levels were present in 6 men with a normal potency and in 6 men with impotence. An association of clearly elevated PRL levels and impotence, has also been found by others (Gura et al., 1980).

In approximately one third of the hyperprolactinemic subjects with renal failure normal levels of LH despite low testosterone levels were observed (Gomez et al., 1980). Treatment with bromocriptine resulted in an increase in basal LH, suggesting an effect of PRL on the pituitary (Gomez et al., 1980). Bommer et al. (1981) showed that administration of bromocriptine to uremic men raised testosterone levels without affecting LH, suggesting a direct action of PRL on gonadal function.

Subnormal levels of DHA were found in 11 men with end-stage renal disease (Zumoff et al., 1980).

Hyperprolactinemia seemed also to be involved in suppression of basal testosterone levels in patients

with acute renal failure (Kokot et al., 1982).

A direct toxic effect of ureum on reproductive functions cannot be ruled out in all these studies.

4. Infertility, impotence and hyperprolactinemia

Hyperprolactinemia may be found in patients with infertility, impotence, hypogonadism and galactorrhea. Numerous studies have been performed to investigate the incidence of hyperprolactinemia and to elucidate the relation between the complaints and hyperprolactinemia. Hyperprolactinemia does not appear to be a common cause of infertility or impotence in the male (Table 1.3).

The incidence of hyperprolactinemia varies widely in these series, which seems to depend on the composition of the group studied and on what is considered to be the normal range for plasma PRL (Hermanns & Hafez, 1981).

5. PRL and seminal plasma

In 1975 PRL was detected in human semen, using a radioimmunoassay (Sheth et al., 1975) and its concentration turned out to be four to seven times higher than in serum. Decreased PRL concentrations have been shown in seminal fluid of oligozoospermic and azoospermic men compared to normal men (Sheth et al., 1975; Koskimies et al., 1978; Merino et al., 1980). Although a positive correlation between semen PRL levels and sperm count could be demonstrated by some authors (Sheth et al., 1974; Biswas et al., 1978; Schoenfeld et al., 1979; Smith et al., 1979;

TABLE 1.3 Incidence of hyperprolactinemia in men with reproductive dysfunctions.

syndrome	incidence %	references	
infertility	40	Boucher et al.,	1977
		Hermabessiere et al.,	1977
	9	Roulier et al.,	1978
	8	Mattei & Roulier,	1977
		Masala et al.,	1979
	4	Segal et al.,	1979
	3	Segal et al.,	1976
	1	Hargreave et al.,	1977
	< 1	Asfour et al.,	1977
		Blacker et al.,	1977
		Koskimies et al.,	1978
		Pierrepoint et al.,	1978
		Rjosk & Schill	1979
		Åbyholm & Molne,	1980
		Gray et al.,	1980
		Laufer et al.,	1981
impotence	16	Ambrosi et al.,	1980
	7	Spark et al.,	1980
	< 1	Krause,	1978
		Rjosk & Schill,	1979
		Miller et al.,	1980

Suchanek & Longhino, 1981), it was not observed by others (Luqman et al., 1979a; Tolis et al., 1979; Küçükkömürcü et al., 1980; Dericks-Tan et al., 1977; Fossati et al., 1979).

PRL added in vitro to semen of fertile donors resulted in an increase of cyclic AMP levels, and in increased utilisation of fructose and the oxydation of glucose by spermatozoa, processes which are essential for the motility of spermatozoa (Shah et al., 1976; Pedron & Giner, 1978; Velazquez-Ramirez et al., 1980).

It is known that there is an association between ATP-ase activity and sperm capacitation, and it is therefore of interest that ATP-ase activity was stimulated by PRL in vitro (Sheth et al., 1979).

In order to investigate the source of PRL in seminal fluid several studies have been done. There is a correlation between PRL concentrations and fructose, which suggests that seminal vesicles are secreting or concentrating PRL (Krause, 1977). Support of this view has come from Segal et al. (1978) who investigated sperm samples obtained by split-ejaculation, and found PRL levels which were higher in the second than in the first fraction. In congenital absence of the vasa and seminal vesicles seminal PRL was markedly depressed (Schoenfeld et al., 1979). Most convincing is the work of Luqman et al. (1979b,c) who studied men before and after undergoing elective vasectomy and concluded that the accessory sex glands were the major source of seminal PRL.
The possible significance of seminal PRL in hyperprolactinemic states is not understood.

6. Therapy

The current management of hyperprolactinemia consists of administration of dopamine-agonistic drugs, operative treatment and radiotherapy (von Werder et al., 1980).
Some studies (Table 1.4) report correction of reproductive dysfunctions by lowering PRL concentrations to normal irrespective of the mode of therapy.

No effect on infertility was seen in 4 hyperprolactinemic men treated with bromocriptine (Madsen et al., 1980). Laufer et al. (1981) reported improvement of sperm motility and conception during treatment of 3 hyperprolactinemic men with bromocriptine.

TABLE 1.4 Normalization of PRL levels and effects on reproductive functions.

1. restoration of libido and potency	Fonzo et al.,	1977
	Saidi et al.,	1977
	Thorner et al.,	1977
	Buvat et al.,	1978
	Carter et al.,	1978
	Franks & Jacobs,	1978
	Nagulesparen et al.,	1979
	Pierini et al.,	1979
	Pont et al.,	1979
2. improvement of gonadotropins	Thorner et al.,	1980a
	Grisoli et al.,	1981
3. increase of testosterone levels	Besser & Thorner,	1976
	Fonzo et al.,	1977
	Hermabessiere et al.,	1977
	Saidi et al.,	1977
	Buvat et al.,	1978
	Carter et al.,	1978
	Nagulesparen et al.,	1979
	Pont et al.,	1979
	Arafah et al.,	1981
4. improvement of sperm qualities	Besser & Thorner,	1976
	Hermabessiere et al.,	1977
	Saidi et al.,	1977
	Nagulesparen et al.,	1979
5. normalization of DHA levels	Vermeulen & Ando,	1978

7. Conclusions and aim of the clinical studies

Most of the studies mentioned suggest effects of PRL excess in male reproduction. No study is conclusive in showing the mechanisms which may be involved in hyperprolactinemic men. Various aberrations in hypothalamic, pituitary and gonadal functions have been described. The purpose of the work described in this thesis was to further investigate some aspects of male hyperprolactinemia.

The following questions may be asked:

1. Does hyperprolactinemia cause loss of libido and/or potency?
2. Does hyperprolactinemia directly interfere with spermatogenesis?
3. Why do a significant number of hyperprolactinemic men have low blood levels of testosterone?
4. Why do hyperprolactinemic men with low testosterone blood levels have normal basal gonadotropin levels and mostly a normal increase during stimulation with LRH?

To answer these questions two groups of patients were investigated. The first group consisted of men with a PRL-secreting pituitary tumor, the second group were men visiting our out-patient department for infertility. The results of this clinical study will be presented in Chapter 3.

8. References

Åbyholm, T. & K. Molne. Serum prolactin and radiological skull examinations in infertile men with azoospermia or severe oligozoospermia. Int. J. Androl. 1980, 3, 229-235.

Ambrosi, B., Travaglini, P., Beck-Peccoz, P., Bara, R., Elli, R., Paracchi, A. & G. Faglia. Effect of sulpiride-induced hyperprolactinemia on serum testosterone response to HCG in normal men. J. Clin. Endocrinol. Metab. 1976, 43, 700-703.

Ambrosi, B., Gaggini, M., Moriondo, P. & G. Faglia. Prolactin and sexual function. JAMA 1980, 244, 2608.

Arafah, B.M., Manni, A., Brodkey, J.S., Kaufman, B., Velasco, M. & H. Pearson. Cure of hypogonadism after removal of prolactin-secreting adenomas in men. J. Clin. Endocrinol. Metab. 1981, 52, 91-94.

Asfour, M., L'Hermite, M., Hedouin-Quincampoix, M. & P. Fossati. Hypogonadism, galactorrhoea and hyper-prolactinaemia: evaluation of pituitary gonadotrophins reserve before and under bromocriptine. Acta Endocrinol. 1977, 84, 738-749.

Baccetti, B., Fraioli, F., Paolucci, D., Selmi, G., Spera, G. & T. Renieri. High prolactin level and double spermatozoa. Gamete Research 1979, 2, 193-199.

Bauer, A.G.C., Wilson, J.H.P. & S.W.J. Lamberts. The kidney is the main site of prolactin elimination in patients with liver disease. J. Clin. Endocrinol. Metab. 1980, 51, 70-73.

Belisle, S. & J. Menard. Adrenal androgen production in hyperprolactinemic states. Fertil. Steril. 1980, 33, 396-400.

Ben-David, M., Danon, A. & F.G. Sulman. Evidence of antagonism between prolactin and gonadotrophin secretion: effect of methallibure on perphenazine-induced prolactin secretion in ovariectomized rats. J. Endocrinol. 1971, 51, 719-725.

Besser, G.M. & M.O. Thorner. Prolactin and gonadal function. Pathol. Biol. 1975, 23, 779-782.

Besser, G.M. & M.O. Thorner. Bromocriptine in the treatment of the hyperprolactinaemia-hypogonadism syndromes. Postgrad. Med. J. 1976, 52, suppl. 1, 64-70.

Biswas, S., Ferguson, K.M., Stedronska, J., Baffoe, G., Mansfield, M.D. & M.H. Kosbab. Fructose and hormone levels in semen: their correlations with sperm counts and motility. Fertil. Steril. 1978, 30, 200-204.

Blacker, C., Asfour, M., Boutemy, J.-J., Gasnault, J.-P., Fossati, P. & M. L'Hermite. Étude de la prolactinémie basale dans la stérilité masculine. Nouv. Presse Med. 1977, 42, 3979-3980.

Bohnet, H.G., Dahlen, H.G., Wuttke, W. & H.P.G. Schneider. Hyperprolactinemic anovulatory syndrome. J. Clin. Endocrinol. Metab. 1975, 42, 132-143.

Bommer, J., Ritz, E., Bommer, G. & E. del Pozo. Increased testosterone in dialyzed men undergoing bromocriptine treatment. Kidney Int. 1981, 20, 144.

Boucher, D., Hermabessiere, J. & M. Doly. Prolactin secretion in infertile men before and after treatment with bromocriptine. Ann. Biol. Anim. Biochem. Biophys. 1977, 17, 483-498.

Boyar, R.M., Kapen, S., Finkelstein, J.W., Perlow, M., Sassin, J.F., Fukushima, D.K., Weitzman, E.D. & L. Hellman. Hypothalamic-pituitary function in diverse hyperprolactinemic states. J. Clin. Invest. 1974, 53, 1588-1598.

Burrow, G.N., Wortzman, G., Rewcastle, N.B., Holgate, R.C. & K. Kovacs. Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. N. Engl. J. Med. 1981, 304, 156-158.

Buvat, J., Asfour, M., Buvat-Herbaut, M. & P. Fossati. Prolactin and human sexual behaviour. In: Progress in prolactin physiology and pathology. Eds. C. Robijn & M. Harper. Elsevier/North Holland Biomedical Press 1978, 317-329.

Carmen Diaz, M. del, Morita, E., Goijman, S., Rettori, V., Romaniello, R. & L. Debeljuk. Pituitary response to luteinizing hormone-releasing hormone during haloperidol-induced hyperprolactinemia. Fertil. Steril. 1981, 35, 626-628.

Carter, J.N. & H.G. Friesen. Hypogonadism in hyperprolactinemic men. Clin. Res. 1976, 24, 656 A.

Carter, J.N., Tyson, J.E., Warne, G.L., McNeilly, A.S., Faiman, C. & H.G. Friesen. Adrenocortical function in hyperprolactinemic women. J. Clin. Endocrinol. Metab. 1977, 45, 973-980.

Carter, J.N., Tyson, J.E., Tolis, G., van Vliet, S., Faiman, C. & H.G. Friesen. Prolactin-secreting tumors and hypogonadism in 22 men. N. Engl. J. Med. 1978, 299, 847-852.

Costello, R.T. Subclinical adenoma of the pituitary gland. Am. J. Pathol. 1936, 12, 205-215.

Cowden, E.A., Ratcliff, W.A., Ratcliff, J.G., Dobbie, J.W. & A.C. Kennedy. Hyperprolactinaemia in renal disease. Clin. Endocrinol. 1978, 9, 241-248.

Davis, J.L. Lowering prolactin level in a hyperprolactinemic man. Responses of luteinizing hormone, follicle stimulating hormone, and testosterone. Arch. Intern. Med. 1982, 142, 146-148.

Dericks-Tan, J.S.E., Bradler, H. & H-D. Taubert. Different concentrations of LH, FSH, PRL and HCG-β in serum and seminal fluid. Acta Endocrinol. 1977, 84, suppl. 208, 24-25.

Derome, P.J., Peillon, F., Bard, R.H., Jedynak, C.P., Racadot, J. & G. Guiot. Adénomes à prolactine: résultats du traitement chirurgical. Nouv. Presse Med. 1979, 8, 577-583.

Dorow, R., Graf, K-J. & R. Horowski. Effects of chronic treatment with metoclopramide and lisuride on serum prolactin and spermatogenesis in healthy volunteers. Acta Endocrinol. 1981, 96, suppl. 240, 68-69.

Drucker, W.D. & R.R. David. Plasma dehydroepiandrosterone sulfate (DHAS) in normals and patients with hyperprolactinemia. In: Adrenal androgens. Eds. A.R. Genazzani et al. Raven Press N.Y. 1980, 89-94.

Eversmann, T., Eichinger, R., Fahlbusch, R., Rjosk, H.K. & K. von Werder. Die Hyperprolaktinaemie beim Mann: Klinik und Therapie. Schweiz. Med. Wochenschr. 1981, 111, 1782-1789.

Facchinetti, F., Inaudi, P. & A.R. Genazzani. Adrenal response to ACTH in hyperprolactinaemic amenorrhoea: effect of bromocriptine treatment. In: Adrenal androgens. Eds. A.R. Genazzani et al. Raven Press N.Y. 1980, 95-101.

Falaschi, P., Frajese, G., Sciarra, F., Rocco, A. & C. Conti. Influence of hyperprolactinaemia due to metoclopramide on gonadal function in men. Clin. Endocrinol. 1978, 8, 427-433.

Fonzo, D., Sivieri, R., Gallone, G., Andriolo, S., Angeli, A. & F. Ceresa. Effect of a prolactin inhibitor on libido, sexual potency and sex hormones in men with mild hyperprolactinaemia, oligospermia and/or impotence. Acta Endocrinol. 1977, 85, suppl. 212, 142.

Fossati, P., Asfour, M., Blacker, C., Boutemy, J.J. & E. Hermand. Serum and seminal gonadotropins in normal and infertile men: correlations with sperm count, prolactinemia and seminal prolactin. Arch. Androl. 1979, 2, 247-252.

Franks S., Jacobs, S. & J.D.N. Nabarro. Prolactin concentrations in patients with acromegaly: clinical significance and response to surgery. Clin. Endocrinol. 1976, 5, 63-69.

Franks, S., Jacobs, H.S., Martin, N. & J.D.N. Nabarro. Hyperprolactinaemia and impotence. Clin. Endocrinol. 1978, 8, 277-287.

Frantz, A.G. Prolactin. N. Engl. J. Med. 1978, 298, 201-207.

Ganong, W.F. Prolactin: a general overview. In: Central and peripheral regulation of prolactin function. Eds. R.M. McLeod & U. Scapagnini. Raven Press N.Y. 1980, 1-10.

Giusti, G., Bassi, F., Forti, G., Giannotti, P., Calabresi, E., Pazzagli, M., Fiorelli, G., Mannelli, M., Misciglia, N. & M. Serio. Effects of prolactin on androgen secretion by the human adrenal cortex. In: Progress in prolactin physiology and pathology. Eds. C. Robijn & M. Harper. Elsevier/North Holland Biomedical Press 1978, 293-303.

Gomez, F., De La Cueva, R., Wauters, J-P. & T. Lemarchand-Beraud. Endocrine abnormalities in patients undergoing long-term hemodialysis. The role of prolactin. Am. J. Med. 1980, 68, 522-530.

Gray, P., Franken, D.R., Slabber, C.F. & G.M. Potgieter. A possible relationship between prolactin and spermatogenesis in humans. Andrologia 1981, 13, 127-130.

Grisoli, F., Vincentelli, F., Jaquet, P., Guibout, M., Hassoun, J. & P. Farnarier. Prolactin secreting adenoma in 22 men. Surg. Neurol. 1980, 13, 241-247.

Grossman, A., Moult, P.J.A., Gaillard, R.C., Delitala, G., Toff, W.D., Rees, L.H. & G.M. Besser. The opioid control of LH and FSH release: effects of a met-enkephalin analogue and naloxone. Clin. Endocrinol. 1981, 14, 41-47.

Gura, V., Weizman, A., Maoz, B., Zevin, D. & M. Ben-David. Hyperprolactinemia: a possible cause of sexual impotence in male patients undergoing chronic hemodialysis. Nephron 1980, 26, 53-54.

Guyda, H., Hwang, P. & H. Friesen. Immunologic evidence for monkey and human prolactin. J. Clin. Endocrinol. Metab. 1971, 32, 120-123.

Hagen, C., Olgaard, K., McNeilly, A.S. & R. Fisher. Prolactin and the pituitary-gonadal axis in male uraemic patients on regular dialysis. Acta Endocrinol. 1976, 82, 29-38.

Hargreave, T.B., Kyle, K.F., Kelly, A.M. & P. England. Prolactin and gonadotrophins in 208 men presenting with infertility. Br. J. Urol. 1977, 49, 747-750.

Hermabessiere, J., Boucher, D. & G. Gaillard. Hyperprolactinémie chez l'homme stérile, action de bromocriptine. Nouv. Presse Med. 1977, 6, 853-854.

Hermanns, U. & E.S.E. Hafez. Prolactin and male reproduction. Arch. Androl. 1981, 6, 95-125.

Hökfelt, T. & K. Fuxe. Effects of prolactin and ergot alkaloids on the tubero-infundibular dopamine (DA) neurons. Neuroendocrinology 1972, 9, 100-122.

Hsu, T.H., Hsu, S.H., Bias, W.B. & J.E. Tyson. Management of male hyperprolactinemic hypogonadism. Am. J. Med. Sci. 1978, 275, 203-208.

Huseman, C.A., Kugler, J.A. & I.G. Schneider. Mechanism of dopaminergic suppression of gonadotropin secretion in men. J. Clin. Endocrinol. Metab. 1980, 51, 209-214.

Hwang, P., Guyda, H. & H. Friesen. A radioimmunoassay for human prolactin. Proc. Natl. Acad. Sci. USA 1971, 68, 1902-1906.

Ito, T. & R. Horton. The source of plasma dihydrotestosterone in man. J. Clin. Invest. 1971, 50, 1621-1627.

Jacobs, H.S., Franks, S., Murray, M.A.F., Hull, M.G.R., Steele, S.J. & J.D.N. Nabarro. Clinical and endocrine features of hyperprolactinaemic amenorrhoea. Clin. Endocrinol. 1976, 5, 439-454.

Jecht, E., Kleissl, H.P. & U. Pache. Short-term increase of sperm output under metoclopramide administration. Int. J. Androl. 1981, 4, 49-54.

Jequier, A.M., Crich, J.C. & I.D. Ansell. Clinical findings and testicular histology in three hyperprolactinemic infertile men. Fertil. Steril. 1979, 31, 525-530.

Jones, D.L., Jacobs, H.S. & V.H.T. James. The relationship between plasma prolactin and dehydroepiandrosterone and dehydroepiandrosterone sulphate levels in patients with hyperprolactinaemia. In: Adrenal androgens. Eds. A.R. Genazzani et al. Raven Press N.Y. 1980, 83-87.

Kandeel, F.R., Rudd, B.T., Butt, W.R., Logan Edwards, R. & D.R. London. Androgen and cortisol responses to ACTH stimulation in women with hyperprolactinaemia. Clin. Endocrinol. 1978, 9, 123-130.

Kirby, R.W., Kotchen, T.A. & E. Douglas Rees. Hyperprolactinemia - a review of recent clinical advances. Arch. Intern. Med. 1979, 139, 1415-1419.

Kokot, F., Mleczko, Z. & A. Pazera. Parathyroid hormone, prolactin and function of the pituitary-gonadal axis in male patients with acute renal failure. Kidney Int. 1982, 21, 84-89.

Koskimies, A.I., Hovatta, O., Ranta, T. & M. Seppala. Serum and seminal plasma prolactin levels in oligospermia. Int. J. Fertil. 1978, 23, 76-78.

Krause, W. Concentration of immunreactive FSH, LH and prolactin in seminal fluid following i.v. application of LH-RH and TRH. Andrologia 1977, 10, 285-290.

Krause, W. Prolaktinspiegel im Serum bei Patienten mit Störungen der Spermatogenese. Hautarzt 1978, 29, 77-81.

Küçükkömürcü, S., Delogne-Desnoeck, J. & C. Robijn. Prolactin and fructose in human seminal fluid. Int. J. Fertil. 1980, 25, 117-121.

Lachelin, G.C.L., Leblanc, H. & S.S.C. Yen. The inhibitory effect of dopamine agonists on LH release in women. J. Clin. Endocrinol. Metab. 1977, 44, 728-732.

Lackritz, R.M. & A. Bartke. The effect of prolactin on androgen response to human chorionic gonadotropin in normal men. Fertil. Steril. 1980, 34, 140-143.

Lamberts, S.W.J., Klijn, J.G.M.. & J.C. Birkenhäger. Prolactine. Ned. Tijdschr. Geneeskd. 1978, 122, 1327-1334.

Lamberts, S.W.J., Timmers, J.M. & F.H. de Jong. The effect of long-term naloxone infusion on the response of gonadotropins to luteinizing hormone-releasing hormone and on plasma estradiol concentration in a patient with a prolactin-secreting pituitary adenoma. Fertil. Steril. 1981, 36, 678-681.

Laufer, N., Yaffe, H., Margalioth, E.J., Livshin, J., Ben-David, M. & J.G. Schenker. Effect of bromocriptine treatment on male infertility associated with hyperprolactinemia. Arch. Androl. 1981, 6, 343-346.

Leblanc, H., Lachelin, G.C.L., Abu-Fadil, S. & S.S.C. Yen. Effects of dopamine infusion on pituitary hormone secretion in humans. J. Clin. Endocrinol. Metab. 1976, 43, 668-674.

Lightman, S.L., Maguire, A.K., Jeffcoate, S.L., McGarrick, G.M. & H.S. Jacobs. Opioid control of gonadtrophin and prolactin secretion in normal subjects, and patients with hyperprolactinaemic amenorrhoea and other disturbances of gonadotrophin secretion. J. Endocrinol. 1980, 87, 63 P.

Lightman, S.L., Jacobs, H.S., Maguire, A.K., McGarrick, G. & S.L. Jeffcoate. Constancy of opioid control of luteinizing hormone in different pathophysiological states. J. Clin. Endocrinol. Metab. 1981, 52, 1260-1263.

Lim, V.S., Kathpalia, S.C. & L.A. Frohman. Hyperprolactinemia and impaired pituitary response to suppession and stimulation in chronic renal failure: reversal after transplantation. J. Clin. Endocrinol. Metab. 1979, 48, 101-107.

Lobo, R.A., Kletzky, O.A., Kaptein, E.M. & U. Goebelsmann. Prolactin modulation of dehydroepiandrosterone sulfate secretion. Am. J. Obstet. Gynaecol. 1980, 138, 632-636.

Luboshitzky, R., Rosen, E., Trestian, S. & I.M. Spitz. Hyperprolactinaemia and hypogonadism in men: response to exogenous gonadotrophins. Clin. Endocrinol. 1979, 11, 217-223.

Luqman, W.A., Matej, L.A. & M.L. Smith. Comparison of prolactin levels in human semen and seminal plasma. J. Endocrinol. 1979a, 81, 131-133.

Luqman, W.A. & M.L. Smith. Seminal immunoreactive prolactin before and after vasectomy. Clin. Endocrinol. 1979b, 10, 213-215.

Luqman, W., Smith, M.L. & S. Plymate. Inherent ranges of seminal prolactin in pre- and postvasectomy subjects. Int. J. Fertil. 1979c, 24, 286-288.

Mckenna, T.J., Glick, A.D., Cobb,Jr., C.A. & L.S. Jacobs. Galactorrhoea and hypogonadism associated with a radiologically-inapparent prolactin-secreting pituitary tumor. Acta Endocrinol. 1978, 87, 225-233.

Madsen, H., Andersen, O. & P. Hansen. Bromocriptine treatment for male infertility. Andrologia 1980, 12, 379-380.

Magrini, G., Ebiner, J.R., Burckhardt, P. & J.P. Felber. Study on the relationship between plasma prolactin levels and androgen metabolism in man. J. Clin. Endocrinol. Metab. 1976, 43, 944-947.

Magrini, G., Iselin, H., Ebiner, J.R. & J.P. Felber. New aspects of androgens, prolactin and ACTH interaction in men. Arch. Androl. 1979, 2, 141-155.

Martikainen, H. & R. Vihko. hCG-stimulation of testicular steroidogenesis during induced hyper- and hypoprolactinaemia in man. Clin. Endocrinol. 1982, 16, 227-234.

Masala, A., Delitala, G., Alagna, S., Devilla, L, Rovasio, P.P. & G. Lotti. Dynamic evaluation of prolactin secretion in patients with oligospermia: effects of treatment with metergoline. Fertil. Steril. 1979, 31, 63-67.

Mattei, A. & R. Roullier. Can an oligospermia be consequent from hyperprolactinemia? Acta Endocrinol. 1977, 85, suppl. 212, 210.

Merino, G., Canales, E.S., Vadillo, M.L., Forsbach, G., Solis, J. & A. Zarate. Abnormal prolactin levels in serum and seminal plasma in infertile men. Arch. Androl. 1980, 4, 353-355.

Metcalf, M.G., Espiner, E.A. & R.A. Donald. Lack of effect of prolactin suppression on plasma dehydroepiandrosterone sulphate. Clin. Endocrinol. 1979, 10, 539-544.

Miller, J.B., Howards, S.S. & R.M. MacLeod. Serum prolactin in organic and psychogenic impotence. J. Urol. 1980, 123, 862-864.

Monroe, S.E., Levine, L, Chang, R.J., Keye, Jr., W.R., Yamamoto, M. & R.B. Jaffe. Prolactin-secreting pituitary adenomas. V. Increased gonadotroph responsivity in hyperprolactinemic women with pituitary adenomas. J. Clin. Endocrinol. Metab. 1981, 52, 1171-1178.

Nagulesparen, M., Ang, V. & J.S. Jenkins. Bromocriptine treatment of males with pituitary tumours, hyperprolactinaemia and hypogonadism. Clin. Endocrinol. 1978, 9, 73-79.

Parker, L.N., Chang, S. & W.D. Odell. Adrenal androgens in patients with chronic marked elevation of prolactin. Clin. Endocrinol. 1978, 8, 1-5.

Pedron, N. & J. Giner. Effect of prolactin on the glycolytic metabolism of spermatozoa from infertile subjects. Fertil. Steril. 1978, 29, 428-430.

Peillon, F., Bard, H., Mowszowicz, I., Cesselin, F., Lagoguey, M. & F. Boyet. Les adénomes à prolactine chez l'homme. Ann. Endocrinol. (Paris) 1979, 40, 73-74.

Pierini, A.A., Sinay, I., Leiderman, S., Damilano, S., Moguilevsky, J.A. & B. Nusimovich. Effects of bromocriptine on prolactin and testosterone levels in male impotence. Int. J. Fertil. 1979, 24, 214-216.

Pierrepoint, C.G., John, B.M., Groom, G.V., Wilson, D.W. & J.G. Gow. Prolactin and testosterone levels in the plasma of fertile and infertile men. J. Endocrinol. 1978, 76, 171-172.

Pont, A., Shelton, R., Odell, W.D. & C.B. Wilson. Prolactin-secreting tumors in men: surgical cure. Ann. Intern. Med. 1979, 91, 211-213.

Pozo, E. del & J. Brownell. Prolactin. I. Mechanisms of control, peripheral actions and modification by drugs. Horm. Res. 1979, 10, 143-172.

Prescott, R.W.G., Johnston, D.G., Kendall-Taylor, P., Crombie, A., Hall, K., McGregor, A. & R. Hall. Hyperprolactinaemia in men - response to bromocriptine therapy. Lancet 1982, 1, 245-248.

Quigley, M.E., Sheehan, K.L., Casper, R.F. & S.S.C. Yen. Evidence for an increased opioid inhibition of luteinizing hormone secretion in hyperprolactinemic patients with pituitary microadenoma. J. Clin. Endocrinol. Metab. 1980, 50, 427-430.

Rjosk, H.-K. & W.-B. Schill. Serum prolactin in male infertility. Andrologia 1979, 11, 297-304.

Ropert, J.F., Quigley, M.E. & S.S.C. Yen. Endogenous opiates modulate pulsatile luteinizing hormone release in humans. J. Clin. Endocrinol. Metab. 1981, 52, 583-585.

Roulier, R., Mattei, A. & P. Franchimont. Prolactin in male reproductive functions. In: Progress in prolactin physiology and pathology. Eds. C. Robyn & M. Harper. Elsevier/North Holland Biomedical Press 1978, 305-316.

Rubin, R.T., Gouin, P.R., Lubin, A., Poland, R.E. & K.M. Pirke. Nocturnal increase of plasma testosterone in men: relation to gonadotropins and prolactin. J. Clin. Endocrinol. Metab. 1975, 40, 1027- 1033.

Rubin, R.T., Poland, R.E. & B.B. Tower. Prolactin-related testosterone secretion in normal adult men. J. Clin. Endocrinol. Metab. 1967, 42, 112-116.

Rubin, R.T., Poland, R.E., Sowers, J.R. & J. Hershman. Influence of methyl-TRH-induced prolactin increase on serum testosterone levels in normal adult men. J. Clin. Endocrinol. Metab. 1978, 46, 830-833.

Saidi, K., Wenn, R.V. & F. Sharif. Bromocriptine for male infertility. Lancet 1977, 1, 250-251.

Schoenfeld, C., Amelar, R.D., Dubin, L. & M. Numeroff. Prolactin, fructose, and zinc levels found in human seminal plasma. Fertil. Steril. 1979, 32, 206-208.

Segal, S., Polishuk, W.Z. & M. Ben-David. Hyperprolactinemic male infertility. Fertil. Steril. 1976, 27, 1425-1427.

Segal, S., Ron, M., Laufer, N. & M. Ben-David. Prolactin in seminal plasma of infertile men. Arch. Androl. 1978, 1, 49-52.

Segal, S., Yaffe, H., Laufer, N. & M. Ben-David. Male hyperprolactinemia: effects on fertility. Fertil. Steril. 1979, 32, 556-561.

Seki, K. & K. Kato. Elevated serum DHEA-S levels in association with hyperprolactinemia. Endocrinol. Japon. 1981, 28, 79-81.

Serri, O., Somma, M., Rasio, E., Beauregard, H. & J. Hardy. Prolactin-secreting pituitary adenomas in males: trans-sphenoidal microsurgical treatment. Can. Med. Assoc. J. 1980, 122, 1007-1013.

Shah, G.V., Desai, R.B. & A.R. Sheth. Effect of prolactin on metabolism of human spermatozoa. Fertil. Steril. 1976, 27, 1292-1294.

Sheth, A.R., Mugatwala, P.P., Shah, G.V. & S.S. Rao. Occurrence of prolactin in human semen. Fertil. Steril. 1975, 26, 905-907.

Sheth, A.R., Gunjikar, A.N. & G.V. Shah. Effect of LH, prolactin and spermine on ATPase activity of human spermatozoa. Andrologia 1979, 11, 11-14.

Smith, M.L., Luqman, W.A. & J.S. Rakoff. Correlations between seminal radioimmunoreactive prolactin, sperm count, and sperm motility in prevasectomy and infertility clinic patients. Fertil. Steril. 1979, 32, 312-315.

Snyder, P.J., Bigdeli, H., Gardner, D.F., Mihailovic, V., Rudenstein, R.S., Sterling, F.H. & R.D. Utiger. Gonadal function in fifty men with untreated pituitary adenomas. J. Clin. Endocrinol. Metab. 1979, 48, 309-314.

Spark, R.F., White, R.A. & P.B. Connolly. Impotence is not always psychogenic. JAMA 1980, 243, 750-755.

Spark, R.F., Wills, C.A., O'Reilly, G.O., Ransil, B.J. & B.J. Bergland. Hyperprolactinaemia in males with and without pituitary macroadenomas. Lancet 1982, 2, 129-132.

Stricker, S. & F. Grueter. Action du lobe antérieur de l'hypopfyse sur la montée laiteuse. C.R. Soc. Biol. (Paris) 1928, 99, 1928.

Suchanek, E. & N. Longhino. The levels of the male serum and seminal fluid prolactin in oligospermia and azoospermia. Int. J. Fertil. 1981, 26, 128-131.

Suominen, J.J.O., Nikkanen, V., Multamaki, S. & M. Hyyppa. Prolactin in azoospermic men and its relation to testicular morphology, serum testosterone and gonadotrophin levels. Andrologia 1979, 11, 15-17.

Thorner, M.O., McNeilly, A.S., Hagan, C. & G.M. Besser. Long-term treatment of galactorrhoea and hypogonadism with bromocriptine. Br. Med. J. 1974, 2, 419-422.

Thorner, M.O., Edwards, C.R.W., Hanker, J.P., Abraham, G. & G.M. Besser. Prolactin and gonadotropin interaction in the male. In: The testis in normal and infertile men. Eds. P. Troen & H.R. Nankin. Raven Press N.Y. 1977, 351-366.

Thorner, M.O. & G.M. Besser. Bromocriptine treatment of hyperprolactinaemic hypogonadism. Acta Endocrinol. 1978, 88, suppl. 216, 131-146.

Thorner, M.O., Martin, W.H., Rogol, A.D., Morris, J.L., Perryman, R.L., Conway, B.P., Howards, S.S., Wolfman, M.G. & R.M. Macleod. Rapid regression of pituitary prolactinomas during bromocriptine treatment. J. Clin. Endocrinol. Metab. 1980a, 51, 438-445.

Thorner, M.O., Rogol, A.D., Evans, W.S., Nunley, Jr., W.C. & R.M. MacLeod. The effects of prolactin on gonadal function in man. In: Central and peripheral regulation of prolactin. Eds. R.M. MacLeod & U. Scapagnini. Raven Press N.Y. 1980b, 271-285.

Turpin, G., Heshmati, H.M., Roger, M., Legrand, S., Guillemant, S. & J.L. de Gennes. La fonction corticosurrénale dans les hyperprolactinêmies. Ann. Endocrinol. (Paris) 1979a, 40, 553-554.

Turpin, G., Heshmati, H.M., Nahoul, K., Wright, F., Mowszowicz, I. & J.L. de Gennes. Étude du role de la prolactine sur le metabolisme des hormones gonadiques par le test a HCG. Ann. Endocrinol. (Paris) 1979b, 40, 423-424.

Varma, M.M., Huseman, C.A., Johanson, A.J. & R.M. Blizzard. Effect of prolactin on adrenocortical and gonadal function in normal men. J. Clin. Endocrinol. Metab. 1977, 44, 760-762.

Velazques-Ramirez, Vilar-Rojas, C. & J.J. Hicks. Similar effects of prolactin and dbcAMP upon human spermatozoa metabolism. Int. J. Androl. 1980, 3, 23-31.

Vermeulen, A., Suy, E. & R. Rubens. Effect of prolactin on plasma DHEA (S) levels. J. Clin. Endocrinol. Metab. 1977, 44, 1222-1225.

Vermeulen, A. & S. Ando. Prolactin and adrenal androgen secretion. Clin. Endocrinol. 1978, 8, 295-303.

Volpê, R., Killinger, D., Bird, C., Clark, A.F. & H. Friesen. Idiopathic galactorrhea and mild hypogonadism in a young adult male. J. Clin. Endocrinol. Metab. 1972, 35, 684-692.

Werder, K. von, Fahlbusch, R. & H.K. Rjosk. Hyperprolaktinaemie. Internist (Berlin) 1977, 18, 520-528.

Werder, K. von, Eversmann, T., Fahlbusch, R. & H.K. Rjosk. Treatment of hyperprolactinemia. In: Central and peripheral regulation of prolactin. Eds. R.M. MacLeod & U. Scapagnini. Raven Press N.Y. 1980, 253-269.

Zumoff, B., Walter, L., Rosenfeld, R.S., Strain, J.J., Degen, K., Strain, G.W., Levin, J. & D. Fukushima. Subnormal plasma adrenal androgen levels in men with uremia. J. Clin. Endocrinol. Metab. 1980, 51, 801-805.

CHAPTER 2

PRL AND REPRODUCTIVE FUNCTIONS IN MALE RATS

1. Introduction

In search of the physiological role of PRL in reproductive functions of male mammals, much attention has been paid to the possible involvement of PRL in the growth of the testes and the accessory sex glands. During sexual development of the male rat PRL levels rise at day 25 and remain relatively constant until day 50. Initiation of the rapid phase of testicular growth at 25 days is correlated with an elevation of serum FSH and PRL levels. Rapid growth of the accessory sex glands begins somewhat later in the presence of high PRL levels, falling FSH levels and gradually increasing LH levels, suggesting a synergism between PRL and LH-stimulated testicular androgens (Negro-Villar et al, 1973). Administration of PRL to hypophysectomized male Sprague-Dawley rats elicited minimal growth of the seminal vesicles, but none of the prostate (Chase et al., 1957). From results of this study a synergism of PRL with testosterone in stimulating growth of male accessory sex glands was suggested.

PRL alone can also influence testicular and/or accessory sex organ function as has been clearly demonstrated in PRL deficient animals (see review Bartke, 1980a). Administration of PRL to dwarf mice, which have PRL deficiency and LH and FSH deficiency, was followed by growth of the testes and the accesso-

ry reproductive glands, increased testicular testosterone production and induced fertility (Bartke et al, 1965, 1966, 1970a-b, 1971, 1975, 1977a-c, 1980a). Recently an increased release of FSH was also observed during treatment with PRL in these PRL deficient animals (Bartke et al., 1981a).
In the male golden hamster, testicular atrophy can be induced by exposure to short photoperiod, which is also accompanied by a decrease of plasma PRL levels. PRL administration to these animals was followed by growth of the testes (Bartke et al., 1975; 1980b) and by an increase of plasma FSH levels (Bartke et al., 1981b).

It can be concluded from these studies that testis growth and testicular functions are regulated by various adenohypophyseal hormones including PRL (Bartke et al., 1978; Lipsett, 1980).

There is evidence that PRL alone, in the absence of testosterone has no effect on prostatic growth and this suggests that the synergistic action of PRL with testosterone is mediated by the action of testosterone (Holland & Lee, 1980).

The next paragraphs will deal with interactions between PRL, gonadotropins and androgens and with the effects of PRL on gonads, accessory sex glands and adrenals in male rats in physiological and hyperprolactinemic conditions.

2. Physiological role of PRL

2.1. Testes

There is some evidence that PRL can enhance the stimulation of the androgen secretion by LH in hypo-

physectomized rats. Both the measurement of androgen secretion and administration of PRL were carried out in different ways.

Hafiez et al., (1971) showed that administration of PRL subcutaneously (s.c.) together with LH to hypophysectomized rats was able to normalize the activity of 3β-hydroxysteroid-dehydrogenase in the testes. It was shown by the same authors (Hafiez et al., 1972a) that treatment of hypophysectomized rats with PRL and LH resulted in an increased conversion of acetate into testosterone in minced testes of these animals, which conversion was enhanced even more than after treatment with LH alone. Treatment with PRL alone had no effect.

By estimating the androgen production by the testes using either plasma testosterone levels (Hafiez et al., 1972b; Bartke & Dalterio, 1976) or sex accessory organ weight, Balin & Schwartz (1972) and Johnson (1974) found that PRL augmented the testicular response to LH when both hormones were given simultaneously.

The presence of ectopic pituitary transplants in hypophysectomized rats caused PRL levels, which were also able to increase testicular sensitivity to LH. Lu et al. (1977) showed that pituitary grafts under the kidney capsule partially prevented atrophy of the testes and ventral prostate. Small amounts of LH and FSH could be detected in peripheral plasma of hypophysecytomized animals with pituitary grafts, while both LH and FSH were undetectable in the plasma of hypophysectomized rats without pituitary grafts, an observation confirmed by Lam et al. (1976).
Furthermore, treatment of the pituitary grafted animals with a dopamine agonistic agent did not alter either LH and FSH levels, or testis and ventral pro-

state weight. This observation suggests that PRL alone had no effect on the testes and the accessory sex glands, but that prevention of testicular atrophy in hypophysectomized animals is due to the presence of small amounts of LH and FSH in peripheral plasma, secreted by the pituitary grafts.

PRL given subcutaneously for over 30 days to hypophysectomized rats had no effect on the maintenance of spermatogenesis (Bartke & Lloyd, 1970b). Administration of PRL to hypophysectomized rats did not increase the reduced numbers of spermatids and pachytene spermatocytes (Sivelle et al., 1978).
The maintenance of spermatogenesis in hypophysectomized rats by ectopic pituitaries (Lu et al., 1977) can also be explained by the presence of small amounts of LH and FSH in peripheral blood as mentioned above.

Some work on the effects of PRL on spermatogenesis have been done in intact rats.
Suppression of PRL levels in intact adult male rats with bromocriptine did not reveal a role for PRL in initiating or maintaining spermatogenesis in the rat (Alger et al., 1975). It was shown by Nag et al. (1981) that suppression of PRL with bromocriptine in immature rats resulted in an inhibition of the conversion of spermatocytes into spermatids.

2.2. Accessory sex glands

Grayhack & Lebowitz (1967) showed that PRL in combination with testosterone stimulated the growth of the lateral lobe of the prostate in hypophysectomized -orchidectomized rats. Implantation of one pituitary under the kidney capsule in castrated, castrated-

adrenalectomized and castrated-hypophysectomized rats was not followed by a rise of the weights of prostate and seminal vesicles (Bartke & Lloyd, 1970a). The weights of dorso-lateral prostates of castrated rats treated with testosterone propionate were significantly heavier when PRL was also administered (Moger et al., 1973). PRL did not increase the effect of testosterone on the weight of the seminal vesicles. Stimulation of the functional activity of the prostate by PRL-testosterone synergism was suggested, since an increase of the zinc content of the dorso-lateral prostate was found.
Treatment with PRL of hypophysectomized-castrated, testosterone-substituted rats significantly increased the nuclear levels of DHT in both the head of the epididymis and ventral prostate (Baker et al., 1977). Other confirmation of PRL-testosterone synergism was the finding of a significant increase of prostatic RNA and DNA concentration in normal and castrated rats after treatment with PRL (Thomas & Manandhar, 1975).

A marked increase in the weight of prostate transplants was seen during treatment of the male host with testosterone together with PRL (Edwards & Thomas, 1980).

Injection of PRL alone to intact male rats caused a small although significant increase of the weight of the dorsal lobes of the prostate (Thomas & Manandhar, 1977).

Furthermore, the finding that PRL alone could increase the uptake of ^{65}Zn by the dorso-lateral prostate of castrated rats indicates an effect of PRL independant of testosterone (Moger et al., 1972).

No augmentation by PRL of the effect of testosterone upon prostates and seminal vesicles in hypophy-

sectomized rats was found by Johnson (1974). Administration of PRL to hypophysectomized rats had also no effect on prostatic weight (Yamanaka et al., 1975). In hypophysectomized rats treated with testosterone prostatic 5α-reductase activity was equal to that in intact controls; when PRL was given together with testosterone 5α-reductase activity was doubled.
When PRL was given (without testosterone) to intact rats 5α-reductase activity did not increase, but decreased (Yamanaka et al., 1975).

These controversial effects of PRL on the accessory sex glands do not justify the opinion that PRL alone or with testosterone has merely a stimulatory effect. Moreover, the results of the experiments in hypophysectomized animals have to be interpreted with caution, since hardly any PRL levels have been mentioned. It has been observed that even very small remnants of pituitary tissue in the sellar fossa after hypophysectomy are able to induce hyperprolactinemia (van Straalen et al., 1981).

3. Hyperprolactinemia

The finding of an association between hyperprolactinemia and hypogonadism in men gave rise to a renewed interest in the possible effects of elevated PRL levels on reproductive functions in male animals.

Implantation of a PRL-secreting tumor into adult male rats resulted in severe testicular atrophy within months (Wilson, 1971; Fang et al., 1974; Hodson et al., 1980). The concentration of testosterone was very much decreased, the weight of the testes declined to less than half of the control value and the

weight of the accessory reproductive glands decreased to one fourth (Fang et al., 1974). Very low levels of serum LH were measured (Hodson et al., 1980).

However, there are remarkable differences between these observations obtained with a pituitary tumor and the results obtained with multiple ectopic grafts of normal pituitary tissue. Both conditions result in hyperprolactinemia, but the level obtained is much lower in the latter situation. Also in that situation testes weight and plasma testosterone levels did not change, whereas seminal vesicles significantly grew larger (Bartke, 1977b).

3.1. Gonadotropins

In 1937 a pronounced antigonadal action of PRL was found in adult pigeons, which was explained by a direct action of the administration of PRL on the pituitary, with suppression of FSH release (Bates et al., 1937).

Both serum LH and FSH levels were decreased when hyperprolactinemia was induced in male rats by two or more pituitary gland transplants under the kidney capsule (McNeilly et al., 1978). An increase in FSH levels was seen in intact rats, made hyperprolactinemic by 2 pituitary grafts (Tresguerres & Esquifino, 1981b).

Injections of PRL, implants of PRL in the median eminence and pituitary grafts under the kidney capsule could partially inhibit the rise of serum LH after castration of male rats (Grandison et al., 1977; Celotti et al., 1978; Winters & Loriaux, 1978; Tresguerres et al., 1981a). Treatment of castrated male rats with PRL injections had no effect on

FSH levels (Celótti et al., 1978).

Another indication that PRL presumably acts directly at the pituitary level, was a diminished response of LH after LRH administration to hyperprolactinemic rats (Gudelsky et al., 1976; Grandison et al., 1977; Winters & Loriaux, 1978; Esquifino & Tresguerres, 1979; Greeley & Kizer, 1979; McNeilly et al., 1980a; Tresguerres & Esquifino, 1981b).

3.2. LRH and dopamine in hyperprolactinemia

The role of PRL in the regulation of LH and FSH secretion is currently of great interest, especially in female rats (Smith, 1980). The mechanism by which high blood levels of PRL lead to suppression of gonadotropin secretion is unknown. Several hypotheses have been proposed (Porter et al., 1980):

1. PRL could act directly on the gonadotropin secreting cells to suppress gonadotropin secretion.
2. The inhibitory effect of PRL on gonadotropin secretion could be mediated by the hypothalamus.

Hyperprolactinemia induced in the rat by a PRL-secreting tumor (MtTW5) or by ectopic pituitary grafts increased the hypothalamic LRH stores and blocked the discharge of LRH from the hypothalamus, which follows castration (Gil-Ad et al., 1978; Grandison et al., 1977). An increase of hypothalamic content of LRH has also been reported in intact male rats bearing a PRL- and growth hormone-secreting tumor MtTW15 (Hodson et al., 1980).

It has been well established that PRL can regulate its own secretion, probably mediated by hypothalamic dopamine, the most important PRL inhibiting factor (Chapter 6).

The increased hypothalamic dopamine turnover in reaction to elevated serum PRL levels (Hökfelt & Fuxe, 1971) has been suggested to inhibit also the release of hypothalamic LRH into the portal vessels. A decrease of LRH in hypophysial portal blood in hyperprolactinemic ovariectomized animals might explain that LH did not rise directly after ovariectomy in these animals (Beck & Wuttke, 1977).
Hyperprolactinemia results in increased dopamine levels in hypophysial portal blood (Cramer et al., 1979), but there is insufficient evidence that this increased release of dopamine can suppress the secretion of LH and FSH.

Some authors reported a decreased pituitary content of LH (McNeilly et al., 1978), whereas an increased content was found by others (Hodson et al., 1980; Lamberts et al., 1981) in hyperprolactinemic rats. Also these findings do not offer an explanation for the low blood levels of LH and FSH in hyperprolactinemic rats.

3.3. Testes and accessory sex glands

Although plasma LH and FSH are suppressed in hyperprolactinemic rats, testosterone levels do not seem to be affected (Bartke et al., 1977a; McNeilly et al., 1978; 1980a; Tresguerres et al., 1981a). These observations are in contrast to those in hyperprolactinemic men who often exhibit normal serum gonadotropin levels and decreased serum testosterone levels (Chapter 1).

Some authors were able to show that PRL has an inhibitory effect on the testes in rats. Serum testos-

terone levels and testosterone secretion by testicular tissue in vitro in response to the administration of hCG in hyperprolactinemic rats were reduced (Tresguerres et al., 1981a; Sharpe et al., 1980a-b). In animal experiments no structural changes in the male accessory sex glands during hyperprolactinemia have been observed (See review Hermanns & Hafez, 1981, Chapter 1).

4. Receptors

4.1. Testes

The presence of specific binding sites for PRL on the testes has been reported by Aragona & Friesen (1975). These receptors were located on Leydig cells (Aragona et al., 1977; Charreau et al., 1977b) and have led to numerous studies on this subject. The finding that PRL was able to stimulate steroidogenic enzymes in the Leydig cells and to initiate spermatogenesis, led many investigators to study the effects of PRL on receptors.

Treatment of hypophysectomized rats with PRL (Zipf et al., 1978) or with one ectopic pituitary (McNeilly et al., 1979) caused a rise of the number of LH receptors in rat testicular tissue (Purvis et al., 1979; Bambino et al., 1980).

The number of LH receptors per Leydig cell in hyperprolactinemia was higher than in control rats (Sharpe et al., 1979, 1980a-b, Chan et al., 1981). LH negatively regulates its Leydig cell receptor (Sharpe & McNeilly, 1979) , and the increase in LH receptor number in hyperprolactinemia may be due to a chronic reduction of LH levels. Increase of LH re-

ceptor binding in the hypophysectomized rat treated with PRL can be explained only by a direct effect of PRL on the Leydig cell. Apparently normal serum concentrations of testosterone are seen in rats hyperprolactinemic through pituitary grafts, which have low serum levels of LH. In rats with a PRL-secreting tumor very low testosterone levels were found (Hodson et al., 1980); in these animals serum LH has also been found to be low.

4.2. Accessory sex glands

PRL binding sites have been demonstrated in the ventral prostate of the male rat (Kledzik et al., 1976; Charreau et al., 1977a), in the dorsal prostate (Aragona et al., 1977) and in the seminal vesicles (Barkey et al., 1977;1979). The presence of testosterone is essential for the maintenance of PRL binding sites in these organs.

5. In vitro studies with PRL

Following reports that PRL can stimulate male accessory sex glands directly, effects of PRL on the conversion of testosterone to DHT have been studied in the cultured ventral prostates of rats. The results of such studies are equivocal. Bovine PRL and ovine PRL had opposite effects on testosterone uptake and metabolism in the rat prostate gland (Lloyd et al., 1973). These differences were probably caused by differences in the degree of purity of the PRL preparations used. PRL did not alter the metabolism of testosterone to DHT (Johansson, 1976a). According

to Manandhar & Thomas (1976) PRL reduced the formation of testosterone in DHT in the rat ventral prostate.

Johansson (1975) found a greater stimulation of the synthesis of RNA and protein by bovine PRL and testosterone than with testosterone alone in tissue cultures of the rat ventral prostate.
Adenylate cyclase activity in prostates was stimulated by ovine PRL (Golder et al., 1972).
PRL was able to increase the specific binding of DHT to the ventral prostate nuclei (Johansson, 1976b)

In some (Thomas & Keenan, 1976) but not all (Datatreymurty et al., 1975) studies, stimulation of the seminal vesicles by PRL has not been observed as consistently as has been shown for the PRL stimulation of the rat prostate.

6. Adrenals

Little consideration has been given to a possible participation of the adrenal in the inhibition of LH and FSH secretion during hyperprolactinemia. It has been found by some workers (Greeley & Kizer, 1979) that the adrenals may participate in the suppression by PRL of the LRH-induced LH-release; others have not been able to confirm these findings (McNeilly et al., 1980a).

It has been known for a long time that PRL exerts a direct effect on the adrenal cortex. Administration of PRL combined with ACTH resulted in blood levels of corticosterone which were about one half of those caused by ACTH alone (Bates et al., 1964). PRL administration in vivo stimulated the production of corticosterone by adrenal slices in vitro and inhi-

TABLE 2.1 Effects of PRL on reproductive functions in the male rat.

1. Physiological effects of PRL	
hypothalamic-pituitary level	- elevation of plasma FSH levels
testes	- growth of testes during puberty - increase of testicular testosterone production; synergism with LH - increase of hydroxy-steroid dehydrogenase - accumulation of esterified cholesterol - increase of number of LH receptors - stimulation of spermatogenesis; synergism with LH
accessory sex glands	- growth of prostate and seminal vesicles during puberty - potentiation of the effects of exogenous androgens on growth of accessory sex glands - increase of zinc-uptake in the prostate - suppression of 5α-reductase activity in the prostate
adrenals	- suppression of 5α-reductase activity
2. Elevated PRL levels	
hypothalamic-pituitary level	- increase of DA in portal venous blood - blockade of hypothalamic LRH-release - increased hypothalamic LRH-content - low levels of LH and FSH
testes	- normal testosterone production - diminished response of testosterone to hCG
adrenals	- participation in the suppression of LRH-induced LH-release
3. Extremely elevated PRL levels	
pituitary	- suppression of plasma LH and FSH
testes	- atrophy; low plasma testosterone
accessory sex glands	- atrophy
copulatory behavior	- suppression (Svare, 1979; Doherty, 1981, Bailey, 1982)

bited the formation of reduced steroid metabolites; both effects were probably due to suppression of 5α-reductase activity (Witorsch & Kitay, 1972; Gustafsson & Stenberg, 1975; Ogle & Kitay, 1979).

The low rate of corticosterone production by isolated adrenal cells of hypophysectomized rats could be partially restored to normal by administration of PRL (Lis et al., 1973).

7. Aims and outlines of experimental studies to be reported in Chapters 4-8

Although the effects of PRL (Table 2.1) on reproductive functions in rats bearing a PRL-secreting tumor and exhibiting high PRL levels are rather uniform, the findings in rats with only mild elevations of serum PRL levels are equivocal and elicit several questions:

1. How can normal levels of testosterone be present in moderately hyperprolactinemic male rats, in the presence of decreased gonadotropin levels?
2. By which mechanisms are gonadotropin levels suppressed?
3. Do the the adrenals have a role in the suppression of gonadotropin secretion in hyperprolactinemic male rats?
4. Can spermatogenesis be suppressed by elevated PRL levels?

To answer these questions hyperprolactinemia was induced by a PRL- and ACTH-secreting transplantable rat pituitary tumor in adult male rats (Chapters 4-6). Local effects of PRL on testicular functions were studied by using an intra-testicular pituitary transplant (Chapter 8).

8. References

Alger, E.A., Pfeiffer, J. & A.V. Boccabella. Reinvestigation of the role of prolactin in male rats using ergocryptine. Anat. Rec. 1975, 181, 299-300.

Aragona, C. & H.G. Friesen. Specific prolactin binding sites in the prostate and testis of rats. Endocrinology 1975, 97, 677-684.

Aragona, C., Bohnet, H.G. & H.G. Friesen. Localization of prolactin binding in prostate and testis: the role of serum prolactin concentration on the testicular LH receptor. Acta Endocrinol. 1977, 84, 402-409.

Bailey, D.J. & J. Herbert. Impaired copulatory behaviour of male rats with hyperprolactinaemia induced by domperidone or pituitary grafts. Neuroendocrinology 1982, 35, 186-193.

Balin, M. & N.B. Schwartz. Effects of prolactin on accessory sex organs in hypophysectomized (hypox) and castrate-hypophysectomized (cast-hypox) male rats. American Zoologist 1972, 12, XIX.

Baker, H.W.G., Worgul, T.J., Santen, R.J., Jefferson, L.S. & C.W. Bardin. Effects of prolactin on nuclear androgens in perfused male accessory sex organs. In: The testis in normal and infertile men. Eds. P. Troen & H.R. Nankin. Raven Press N.Y. 1977, 379-385.

Bambino, T.H., Schreiber, J.R. & A.J.W. Hsueh. Gonadotropin releasing hormone and its agonist inhibit testicular luteinizing hormone receptor and steroidogenesis in immature and adult hypophysectomized rats. Endocrinology 1980, 107, 908-917.

Barkey, R.J., Shani, J., Amit, T. & D. Barzilai. Specific binding of prolactin to seminal vesicle, prostate and testicular homogenates of immature, mature and aged rats. J. Endocrinol. 1977, 74, 163-173.

Barkey, R.J., Shani, J. & D. Barzilai. Regulation of prolactin binding sites in the seminal vesicle, prostate gland, testis and liver of intact and castrated adult rats: effect of administration of testosterone, 2-bromo-α-ergocryptine and fluphenazine. J. Endocrinol. 1979, 81, 11-18.

Bartke, A. Influence of luteotrophin on fertility of dwarf mice. J. Reprod. Fertil. 1965, 10, 93-103.

Bartke, A. Influence of prolactin on male fertility in dwarf mice. J. Endocrinol. 1966, 35, 419-420.

Bartke, A. & C.W. Lloyd. The influence of pituitary homografts on the weight of the accessory reproductive organs in castrated male mice and rats and on mating behaviour in male mice. J. Endocrinol. 1970a, 46, 313-320.

Bartke, A. & C.W. Lloyd. Influence of prolactin and pituitary isografts on spermatogenesis in dwarf mice and hypophysectomized rats. J. Endocrinol. 1970b, 46, 321-329.

Bartke, A. Effects of prolactin and luteinizing hormone on the cholesterol stores of the mouse testis. J. Endocrinol. 1971, 49, 317-324.

Bartke, A., Croft, B.T. & S. Dalterio. Prolactin restores plasma testosterone levels and stimulates testicular growth in hamsters exposed to short day-length. Endocrinology 1975, 97, 1601-1604.

Bartke, A. & S. Dalterio. Effects of prolactin on the sensitivity of the testis to LH. Biol. Reprod. 1976, 15, 90-93.

Bartke, A., Smith, M.S., Michael, S.D., Peron, F.G. & S. Dalterio. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977a, 100, 182-186.

Bartke, A. Prolactin and the physiological regulation of the mammalian testis. In: The testis in normal and infertile men. Eds. P. Troen & H.R. Nankin. Raven Press N.Y. 1977b, 367-378.

Bartke, A., Goldman, B.D., Bex, F. & S. Dalterio. Effects of prolactin (PRL) on pituitary and testicular function in mice with hereditary PRL deficiency. Endocrinology 1977c, 100, 1760-1766.

Bartke, A., Hafiez, A.A., Bex, F.J. & S. Dalterio. Hormonal interactions in regulation of androgen secretion. Biol. Reprod. 1978, 18, 44-54.

Bartke, A. Role of prolactin in reproduction in male mammals. Fed. Proc. 1980a, 39, 2577-2581.

Bartke, A., Goldman, B.D., Bex, F.J., Kelch, R.P., Smith, M.S., Dalterio, S. & P.C. Doherty. Effects of prolactin on testicular regression and recrudescence in the golden hamster. Endocrinology 1980b, 106, 167-172.

Bartke, A., Siler-Khodr, T.M. & F. Bex. Effects of prolactin (PRL) on gonadotropin release in mice with congenital PRL deficiency. Experienta 1981a, 37, 530-531.

Bartke, A., Siler-Khodr, T.M., Hogan, M.P. & P. Roychoudhury. Ectopic pituitary transplants stimulate synthesis and release of follicle-stimulating hormone in golden hamsters. Endocrinology 1981b, 108, 133-139.

Bates, R.W., Riddle, O. & E.L. Lahr. The mechanism of the antigonad-action of prolactin in adult pigeons. Am. J. Physiol. 1937, 119, 610-614.

Bates, R.W., Milkovic, S. & M M. Garrison. Effects of prolactin, growth hormone and ACTH, alone and in combination, upon organ weights and adrenal function in normal rats. Endocrinology 1964, 74, 714-723.

Beck, W. & W. Wuttke. Desensitization of the dopaminergic inhibition of pituitary luteinizing hormone release by prolactin in ovariectomized rats. J. Endocrinol. 1977, 74, 67-74.

Celotti, F., Massa, R. & L. Martini. Effect of prolactin on L.H. release in male rats. Neuroendocrinology 1978, 26, 41-49.

Chan, V., Katikineni, M., Davies, T.F. & K.J. Catt. Hormonal regulation of testicular luteinizing hormone and prolactin receptors. Endocrinology 1981, 108, 1607-1612.

Charreau, E.H., Attramadal, A., Torjisen, P.A., Calandra, R., Purvis, K. & V. Hansson. Androgen stimulation of prolactin receptors in rat prostate. Mol. Cell. Endocrinol. 1977a, 7, 1-7.

Charreau, E.H., Attramadal, A., Torjisen, P.A., Purvis, K., Calandra, R. & V. Hansson. Prolactin binding in rat testis: specific receptors in interstitial cells. In: The testis in normal and infertile man. Eds. P. Troen & H.R. Nankin. Raven Press N.Y. 1977b, 387-393.

Chase, M.D., Geschwind, I.I., Bern, H.A. & C.H. Li. Synergistic role of prolactin in response of male sex accessories to androgen. Proc. Soc. Exp. Biol. Med. 1957, 94, 680-683.

Cramer, O.M., Parker, C.R. & J.C. Porter. Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinology 1979, 105, 636-640.

Dattatreymurty, B., Raghavan, V.P., Purandare, T.V., Seth, A.R. & S S. Rao. Synergistic action of prolactin with HCG on rat ventral prostate. J. Reprod. Fertil. 1975, 44, 555-557.

Doherty, P.C., Bartke, A. & M.S. Smith. Differential effects of bromocriptine treatment on LH release and copulatory behavior in hyperprolactinemic male rats. Horm. Behav. 1981, 15, 436-450.

Edwards, W.D. & J.A. Thomas. Morphologic and metabolic characteristics of ventral, lateral, dorsal and anterior prostate transplants in rats. Horm. Res. 1980, 13, 28-39.

Esquifino, A. & J.A.F. Tresguerres. Plasma LH responses in hyperprolactinemic male rats to LHRH before and after bromocriptine treatment and castration. Acta Endocrinol. 1979, 91, suppl. 225, 141.

Fang, V.S., Refetoff, S. & R.L. Rosenfield. Hypogonadism induced by a transplantable, prolactin producing-tumor in male rats: hormonal and morphological studies. Endocrinology 1974, 95, 991-998.

Gil-Ad, J., Locatelli, V., Cocchi, D., Carminati, R., Arezzini, C. & E.E. Mueller. Effect of hyperprolactinemia and 2-Br-α-ergocryptine on neuroendocrine mechanism (s) for gonadotropin control. Life Sci. 1978, 23, 2245-2256.

Golder, M.P., Boyns, A.R., Harper, M.E. & K. Griffiths. An effect of prolactin on prostatic adenylate cyclase activity. Biochem. J. 1972, 128, 725-727.

Grandison, L., Hodson, C., Chen, H.T., Advis, J., Simpkins, J. & J. Meites. Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology 1977, 23, 312-322.

Grayhack, J.T. & J.M. Lebowitz. Effect of prolactin on citric acid of lateral lobe of prostate of sprague-dawley rat. Invest. Urol. 1967, 5, 87-94.

Greeley, G.H. & J.S. Kizer. Evidence for adrenal involvement in the modulatory role of prolactin in luteinizing hormone secretion in the male rat. Endocrinology 1979, 104, 948-953.

Gudelsky, G.A., Simpkins, J., Mueller, G.P., Meites, J. & K.E. Moore. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 1976, 22, 206-215.

Gustafsson, J. & A. Stenberg. Influence of prolactin on the metabolism of steroid hormones in rat liver and adrenals. Acta Endocrinol. 1975, 78, 545-553.

Hafiez, A.A., Philpott, J.E. & A. Bartke. The role of prolactin in the regulation of testicular function: the effect of prolactin and luteinizing hormone on 3β-hydroxysteroid dehydrogenase activity in the testes of mice and rats. J. Endocrinol. 1971, 50, 619-623.

Hafiez, A.A., Bartke, A. & C.W. Lloyd. The role of prolactin in the regulation of testis function: the synergistic effects of prolactin and luteinizing hormone on the incorporation of [1-^{14}C] acetate into testosterone and cholesterol by testes from hypophysectomized rats in vitro. J. Endrocrinol. 1972a, 53, 223-230.

Hafiez, A.A., Lloyd, C.W. & A. Bartke. The role of prolactin in the regulation op testis function: the effects of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectomized rats. J. Endocrinol. 1972b, 52, 327-332.

Hodson, C.A., Simpkins, J.W., Pass, K.A., Aylsworth, C.F., Steger, R.W. & J. Meites. Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinology 1980, 30, 7-10.

Hökfelt, T. & K. Fuxe. Effects of prolactin and ergot alkaloids on the tubero-infundibular dopamine (DA) neurons. Neuroendocrinology 1972, 9, 100-122.

Holland, J.M. & C. Lee. Effects of pituitary grafts on testosterone stimulated growth of rat prostate. Biol. Reprod. 1980, 22, 351-355.

Johansson, R. RNA, protein and DNA synthesis stimulated by testosterone, insulin and prolactin in the rat ventral prostate cultured in chemically defined medium. Acta Endocrinol. 1975, 80, 761-774.

Johansson, R. Effect of some synandrogens and antiandrogens on the conversion of testosterone to dihydrotestosterone in the cultured rat ventral prostate. Acta Endocrinol. 1976a, 81, 398-408.

Johansson, R. Effect of prolactin, growth hormone and insulin on the uptake and binding of dihydrotestosterone to the cultured rat ventral prostate. Acta Endocrinol. 1976b, 81, 854-864.

Johnson, D.C. Temporal augmentation of LH by prolactin in stimulation of androgen production by the testes of hypophysectomized male rats. Proc. soc. exp. biol. med. 1974, 145, 610-613.

Kledzik, G.S., Marshall, S., Campbell, G.A., Gelato, M. & J. Meites. Effects of castration , testosterone, estradiol, and prolactin on specific prolactin-binding activity in ventral prostate of male rats. Endocrinology 1976, 98, 373-379.

Lam, P.C.O., Morishige, W.K. & I. Rothchild. Venous outflow of the hormones secreted by the rat pituitary autotransplanted beneath the kidney capsule. Proc. Soc. Exp. Biol. med. 1976, 152, 615-617.

Lamberts, S.W.J., Zuiderwijk, J.M., Bons, E.G., Uitterlinden, P. & F.H. de Jong. Gonadotropin secretion in rats bearing a prolactin-secreting pituitary tumor: effects of naloxone administration. Fertil. Steril. 1981, 35, 557-562.

Lipsett, M.B. Physiology and pathology of the leydig cell. N. Engl. J. Med. 1980, 303, 682-688.

Lis, M , Gilardeau, C. & M. Chretien. Effect of prolactin on corticosterone production by rat adrenals. Clin. Res. 1973, 21, 1027.

Lloyd, J.W., Thomas, J.A. & M.G. Mawhinney. A difference in the in vitro accumulation and metabolism of testosterone -1,2-^{3}H by the rat prostate gland following incubation with ovine or bovine prolactin. Steroids 1973, 22, 473-483.

Lu, K.H., Grandison, L., Huang, H.H., Marshall, S. & J. Meites. Relation of gonadotropin secretion by pituitary grafts to spermatogenesis in hypophysectomized male rats. Endocrinology 1977, 100, 380-386.

McNeilly, A.S., Sharpe, R.M., Davidson, D.W. & H.M. Fraser. Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 1978, 79, 59-68.

McNeilly, A.S., de Kretser, D.M. & R.M Sharpe. Modulation of prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion by LHRH and bromocriptine (CB154) in the hypophysectomized pituitary-grafted male rat and its effect on testicular LH receptors and testosterone output. Biol. Reprod. 1979, 21, 141-147.

McNeilly, A.S., Sharpe, R.M. & H.M. Fraser. Effect of adrenalectomy or castration on the inhibition of gonadotrophin secretion induced by hyperprolactinaemia in the adult male rat. J. Endocrinol. 1980a, 85, 83-92.

Manandhar, M.S.P. & J.A. Thomas. Effect of prolactin on the metabolism of androgens by the rat ventral prostate gland in vitro. Invest. Urol. 1976, 14, 20-22.

Moger, W.H., Geschwind, I.I. & H.H. Cole. The action of prolactin on the sex accessory glands of the male rat. Proc. Soc. Exp. Biol. Med. 1972, 141, 1017-1021.

Nag, S., Sanyal, S., Ghosh, K.K. & N.M Biswas. Prolactin suppression and spermatogenic developments in maturing rats. A quantitative study. Horm. Res. 1981, 15, 72-77.

Negro-Vilar, A., Krulich, L. & S.M. McCann. Changes in serum prolactin and gonadotropins during sexual development of the male rat. Endocrinology 1973, 93, 660-664.

Ogle, T.F. & J.I. Kitay. Interactions of prolactin and adrenocorticotropin in the regulation of adrenocortical secretion in female rats. Endocrinology 1979, 104, 40-44.

Porter, J.C., Nansel, D.D., Gudelsky, G.A., Foreman, M.M , Pilotte, N.S., Parker, Jr., C.R., Burrows, G.H., Bates, G.W. & J.D. Madden. Neuroendocrine control of gonadotropin secretion. Fed. Proc. 1980, 39, 2896-2901.

Purvis, K., Clausen, O.P.F., Olsen, A., Haug, E. & V. Hansson. Prolactin and leydig cell responsiveness to LH/hCG in the rat. Arch. Androl. 1979, 3, 219-230.

Sharpe, R.M. & A.S. McNeilly. The effect of induced hyperprolactinaemie on leydig cell function and LH-induced loss of LH-receptors in the rat testis. Mol. Cell. Endocrinol. 1979, 16, 19-27.

Sharpe, R.M., McNeilly, A.S., Davidson, D.W. & I.A. Swanston. Leydig cell function in hyperprolactinaemic adult rats. J. Endocrinol. 1980a, 87, 28 P.

Sharpe, R.M. & A.S. McNeilly. Differences between dispersed leydig cells and intact testes in their sensitivity to gonadotrophin-stimulation in vitro after alteration of LH-receptor numbers. Mol. Cell. Endocrinol. 1980b, 18, 75-86.

Sivelle, P.C., McNeilly, A.S. & P.M. Collins. A comparison of the effectiveness of FSH, LH and prolactin in the re-initiation of testicular function of hypophysectomized and estrogen-treated rats. Biol. Reprod. 1978, 17, 878-885.

Smith, M.S. Role of prolactin in regulating gonadotropin secretion and gonad function in female rats. Fed. Proc. 1980, 39, 2571-2576.

Straalen, R.J.C. van, Leemborg, F.G., Vreeburg, J.T.M. & G.H. Zeilmaker. Prolonged steroidogenesis in luteinized ovaries of hypophysectomized rats. Acta Endocrinol. 98, 437-440, 1981.

Svare, B., Bartke, A., Doherty, P., Mason, I, Michael, S.D. & M.S. Smith. Hyperprolactinemia suppresses copulatory behavior in male rats. Biol. Reprod. 1979, 21, 529-535.

Thomas, J.A. & M. Manandhar. Effects of prolactin and/or testosterone on nucleic acid levels in prostate glands of normal and castrated rats. J. Endocrinol. 1975, 65, 149-150.

Thomas, J.A. & E.J. Keenan. Prolactin influences upon androgen action in male accessory sex organs. In: Advances in sex hormone research vol 2. Eds. R.L. Singhal & J.A. Thomas. University Park Press 1976, 425-470.

Thomas, J.A. & M.S.P. Manandhar. Effects of prolactin on the dorsolateral lobe of the rat prostate gland. Invest. Urol. 1977, 14, 398-399.

Tresguerres, J.A.F., Esquifino, A.I., Perez Mendez, L.F. & A. Lopez-Calderon. Possible role of prolactin in the inhibitory effects of estradiol on the hypothalamic-pituitary-testicular axis in the rat. Endocrienology 1981a, 108, 83-87.

Tresguerres, J.A.F. & A.I. Esquifino. Dissociation in the regulation of luteinizing hormone and follicle-stimulating hormone in a hyperprolactinaemic rat model: interrelationships between gonadotrophin and prolactin control. J. Endocrinol. 1981b, 90, 41-51.

Vasquez, J.M., Ellegood, J.O., Nazian, S.J. & V.B. Mahesh. Effect of hyperprolactinemia on pituitary sensitivity to luteinizing hormone-releasing hormone following manipulation of sex steroids. Fertil. Steril. 1980, 33, 543-549.

Wilson, J.T Altered rat hepatic drug metabolism after implantation of a pituitary mammotropic tumor (MtT), Walker carcinosarcoma or adenocarcinoma and after removal of the MtT. Endocrinology 1971, 88, 185-194.

Winters, S.J. & D.L. Loriaux. Suppression of plasma luteinizing hormone by prolactin in the male rat. Endocrinology 1978 , 102, 864-868.

Witorsch, R.J. & J.I. Kitay. Pituitary hormones affecting adrenal 5α-reductase activity: ACTH, growth hormone and prolactin. Endocrinology 1972, 91, 764-769.

Yamanaka, H., Kirdani, R.Y., Saroff, J., Murphy, G.P. & A.A. Sandberg. Effects of testosterone and prolactin on rat prostatic weight, 5α-reductase, and arginase. Am. J. Physiol. 1975, 229, 1102-1109.

Zipf, W.B., Payne, A.H., Kelch, R.P. Prolactin, growth hormone, and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. Endocrinology 1978, 103, 595-600.

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CHAPTER 3

ASPECTS OF INFERTILITY AND PITUITARY FUNCTIONS OF 32 MEN WITH UNTREATED PRL-SECRETING MACRO- AND MICROADENOMAS

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1. Introduction

There is considerable evidence that infertility in female patients can be a consequence of hyperprolactinemia. In contrast to women, whose clinical manifestations - galactorrhea and amenorrhea - lead to early medical consultation, many hyperprolactinemic male patients present with headache and visual impairment due to extrasellar extension of a pituitary tumor (Carter et al., 1978; Derome et al., 1979; Grisoli et al., 1980; Serri et al., 1980). Retrospectively, however, loss of libido, impotence, galactorrhea and sometimes infertility may be present for an extended period (Carter et al., 1978; Franks et al., 1978; Nagulesparen et al., 1978; Thorner et al., 1978). Although hyperprolactinemia obviously seems to exert a suppressive action on reproductive functions in men, the incidence of hyperprolactemia among men with impotence (Miller et al., 1980; Spark et al., 1980) or infertility (Hargreave et al., 1977; Segal et al. 1979; Abyholm et al., 1980; Laufer et al., 1981) has been reported to be very low. Moreover, it has not yet been established whether the symptoms in hyperprolactinemic men are due to the

elevated PRL levels itself, to the accompanying hypogonadism or to compression and/or local destruction of the sellar region by the pituitary tumor with loss of normal pituitary function (Hermanns & Hafez, 1981; Perryman et al., 1981).

Serum gonadotropin concentrations are reported to be normal in hyperprolactinemic men with microadenomas despite decreased serum testosterone levels (Carter et al., 1979; Franks et al., 1978; Thorner et al., 1978; Spark et al., 1982) and they appear to respond normally to LRH (Carter et al., 1978; Franks et al., 1978; Prescott et al., 1982). In contrast, decreased basal serum gonadotropin levels and a blunted response to LRH have been reported in men with PRL-secreting macroadenomas of the pituitary (Nagulesparen et al., 1978; Grisoli et al., 1980; Eversmann et al., 1981; Hermanns & Hafez, 1981). Assessment of the other anterior pituitary functions also revealed pituitary insufficiency in this type of patient (Klijn et al., 1980).

Azoospermia, oligozoospermia, normozoospermia and polyzoospermia have all been reported in hyperprolactinemic men (Snyder et al., 1979); however, little is known about fertility in these men.

The aim of the present study was to investigate the mechanism by which elevated PRL levels might attribute to infertility and/or altered sperm qualities in relation to tumor size, and other pituitary functions. Moreover, the effects of normalization of PRL levels on fertility were studied.

2. Patients and methods

Thirty-two men with hyperprolactinemia were stu-

died. All conditions and medications that have been recognized as principal causes of functional hyperprolactinemia were excluded. They were divided in two groups according to their symptoms. In the first group (group I) these symptoms consisted exclusively of visual disturbances, headache or symptoms of hypopituitarism and clinical evidence of the presence of a pituitary tumor was obtained in all 19 patients. Group II consisted of thirteen out of a group of 598 patients attending our department of male infertility.

The clinical work-up consisted of a detailed history and physical examination. All patients underwent frontal and lateral plain skull radiography, lateral polytomography of the sellar region at intervals of 2.5 mm, and when indicated a computerized axial tomography (CAT) of the suprasellar region and/or pneumoencephalography in order to visualize suprasellar and parasellar extension of the pituitary tumor. Visual fields were plotted with a Goldman apparatus. Ophthalmologic evaluation was completed with fundoscopy and visual acuity. Basal PRL levels and the PRL response to the intravenous administration of 200 microgram thyrotropin-releasing hormone (TRH) (Hoechst, Amsterdam, The Netherlands) was measured in all patients. Anterior pituitary function was assessed by the determination of serum testosterone levels and the response of serum LH and FSH to an intravenous bolus of 100 microgram LRH (Hoechst); serum thyroxine (T_4) and thyroid-stimulating hormone (TSH) response to 200 microgram TRH; plasma 11-deoxycortisol (Compound S) after administration of 6 x 750 mg metyrapone orally in 24 hours. For comparison LRH was also given to nine volunteers and

blood was sampled. When it was possible to obtain an ejaculate, semen analysis was performed.

To investigate the question, whether lowering of serum PRL levels would restore fertility, 11 patients of group II were treated with bromocriptine.

2.1. Hormone assays

Plasma PRL levels were determined by radioimmunoassay, using a commercial kit purchased from IRE (Fleurus, Belgium). Normal values range up to 12 μg/l in normal men. The normal PRL response to TRH has previously been described (maximal increment: 22.9 $\pm$ 17.6 μg/l; % increase: range 102 to 1646%; Klijn et al., 1981).

Serum testosterone levels were measured by RIA (normal values: 0.35 to 0.80 μg/dl) (Verjans et al., 1973).

Serum LH and FSH were estimated by a double antibody RIA, using standard preparations WHO 69/104. In our assay system for LH, 1 U of the WHO standard 69/104 corresponds to 3.8 U of the WHO standard 68/40. Basal levels of LH and FSH varied from 0.6 - 2.5 U/l and from 0.7 - 3.3 U/l respectively (Klijn et al., 1980).

Serum T_4 and TSH were measured by radioimmunoassay techniques as described before (Klijn et al., 1980; Visser et al., 1975). Normal basal levels of T_4 and TSH were 60 - 140 nMol/l and < 1 - 4.9 mU/l respectively. An increase of TSH of at least 5 mU/l has been accepted to be a normal reaction 30 minutes after an i.v. bolus of TRH.

Compound S (normal values after metyrapone ad-

ministration >10 μg/dl) was measured by competitive protein binding assay (Meikle et al., 1969).

2.2. Statistical procedures

Values are given as the mean ± S.E.M. TRH and LRH tests were subjected to an analysis of variance. When significant overall effects were obtained, comparisons between or within groups were made using Duncan's multiple range test. Otherwise a non-parametric test (Mann-Whitney U) was used to establish differences between the two groups. Differences were considered to be significant when $P<0.05$.

3. Results

The mean age of group I (41.4 years: range 15 to 64 years) was significantly different ($P<0.005$) from group II (30.3 years: range 24 to 54 years).
The presenting symptoms are summarized in Table 3.1.

TABLE 3.1 Clinical features of 32 untreated hyperprolactinemic men referred with complaints of a pituitary tumor (group I) and infertility (group II).

	group I		group II	
	n	%	n	%
headache	10	53	-	
visual impairment	8	42	-	
impotence	12	67	-	
loss of libido	9	56	2	15[a]
galactorrhea	3	16	2	15
panhypopituitarism	4	21	-	
infertility	n.k.[b]		13	100
total	19		13	

[a] diminished libido [b] not known

TABLE 3.2 PRL levels (µg/l) and (% increase of PRL) of 19 hyperprolactinemic men with macroadenomas (group I) and 13 hyperprolactinemic infertile men (group II) and their reaction to 200 µg TRH.

	basal	10	20	30	60	120 minutes
group I						
mean	982[a]	1159 (123)[a]	1224 (136)[a]	1361 (145)	1234 (140)	1009 (112)
S.E.M.	294	300 (10)	365 (12)	418 (16)	397 (16)	342 (13)
group II						
mean	42	67 (182)	66 (174)	57 (145)	55 (130)	41 (99)
S.E.M.	8	10 (22)	12 (18)	10 (12)	12 (8)	8 (3)

[a] $P < 0.05$ vs. group II

All patients in group I had macroadenomas, with suprasellar extension in 68% and bone destruction of the sellar fossa in 58% of the patients. In 67% of these patients impotence was noted. Only 2 patients in group II showed enlargement of the sella turcica, one with suprasellar extension. Interesting in this respect was the presence of diminished libido in these 2 patients. The sella turcica was asymmetrical in 54% and normal in 31% of the patients of group II. Basal PRL levels in group I (982 $\pm$ 294 μg/l) were significantly (P<0.005) higher than in group II (42 $\pm$ 8 g/l).

The absolute TRH stimulated PRL levels and the % increase (% of initial value) of PRL are given in Table 3.2. In both groups PRL levels increased after stim-

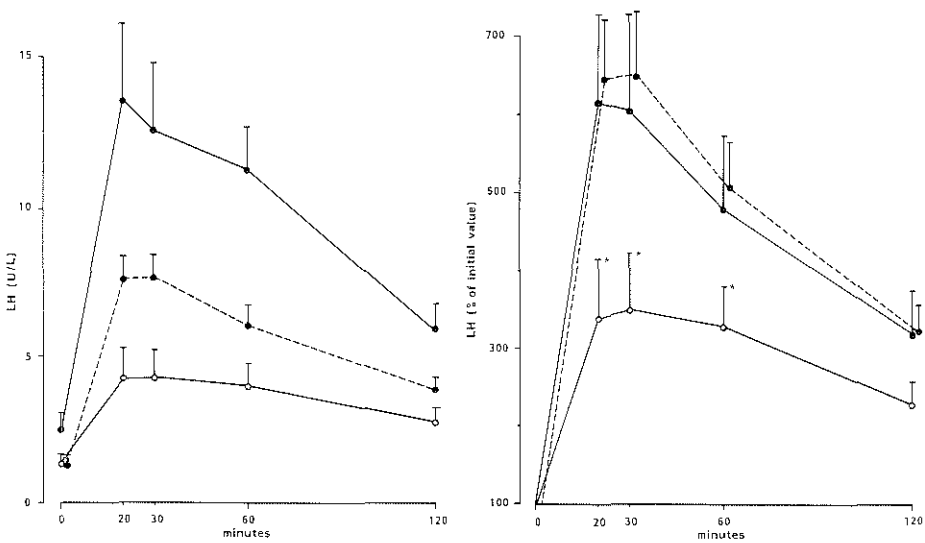

Fig. 3.1 Basal serum LH levels and their response to 100 µg LRH i.v. in 19 hyperprolactinemic men with macroadenomas (o-o), 13 hyperprolactinemic infertile men (●-●) and 9 controls (●····●). Absolute LH levels (left) and % increase of LH (right) are given as mean ± S.E.M. p<0.05 vs. control and group II.

ulation with TRH. The % increase of PRL in group I was significantly less than in group II.

Serum testosterone levels in group I (0.20 ± 0.04 μg/dl) were significantly (P<0.005) lower than in group II (0.40 ± 0.04 μg/dl). Testosterone levels less than 35 μg/dl were noted in 15 patients of group I, but in only 5 patients in group II.

Basal and LRH stmulated LH levels are given in Figure 3.1. There was no difference in basal levels of LH between the control group and group I or group II. However, basal levels of LH in group II were significantly higher than in group I. All groups showed an increase of LH after LRH.

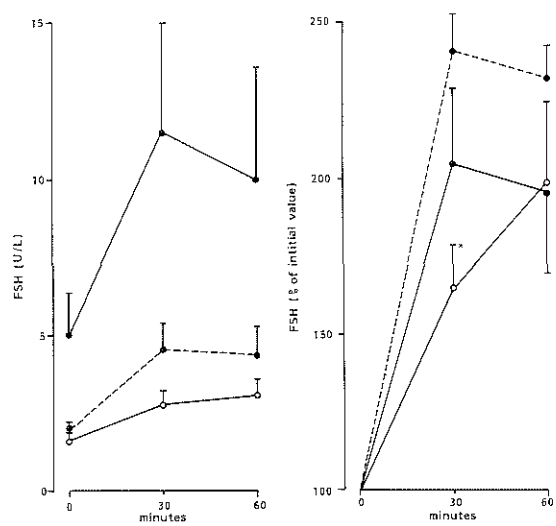

Fig. 3.2 Basal serum FSH levels and their response to 100 μg LRH i.v. in 19 hyperprolactinemic men with macroadenomas (o-o), 13 hyperprolactinemic infertile men (●–●) and 9 controls (●···●). Absolute FSH levels (left) and % increase of FSH (right) are given as mean ± S.E.M. p<0.05 vs. control.

There was no difference between group II and the control group with regard to the % increase of LH, which was significantly less in group I. The absolute maximal increment of LH was also significantly decreased in group I ($P<0.025$).

Basal levels of FSH either in group I or group II were not different from those in the control group, although basal levels of FSH in group II were significantly higher than in group I. Administration of LRH resulted in an increase of FSH in all groups (Figure 3.2).

In group I the % increase of FSH was only 30 minutes after stimulation lower than in the control group.

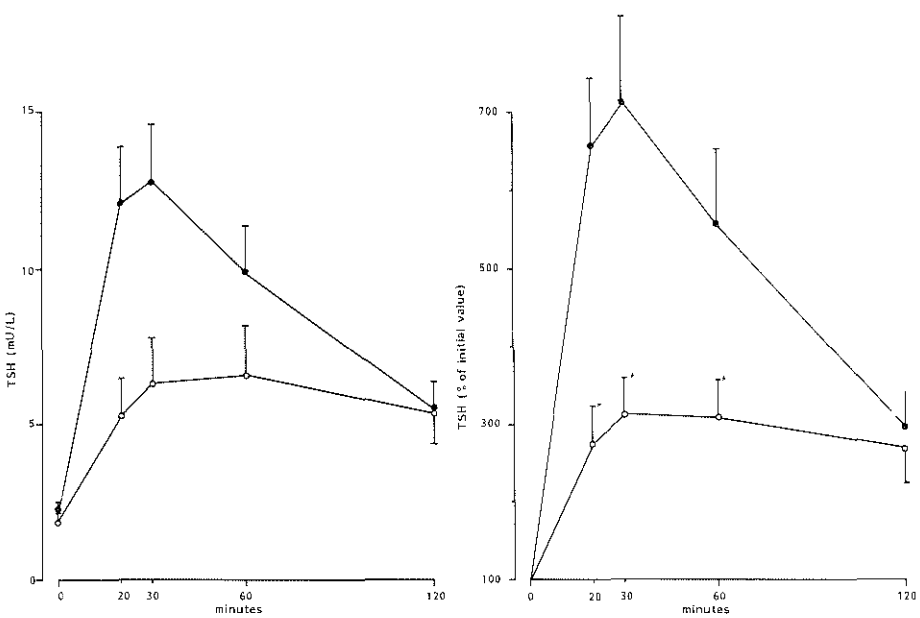

Fig. 3.3 TSH levels (left) and % increase of TSH (right) in response to an i.v. bolus of 200 µg TRH in 19 hyperprolactinemic men with macroadenomas (o–o) and 13 hyperprolactinemic infertile men (●–●). Values are given as mean ± S.E.M.
$p<0.05$

The absolute maximal increment of FSH in group I was significantly less compared to the control group ($P<0.005$) and group II ($P<0.001$).

Seven patients in group I showed a decreased level of T_4, against none of the patients in group II. The mean level of T_4 in group I (72.9 $\pm$ 7.5 nMol/l) was significantly lower than in group II (102 $\pm$ 8.8 nMol/l). Although the levels of TSH significantly increased after TRH administration (Figure 3.3) in group I and group II, the % increase of TSH in group I was significantly less than in group II. A blunted response of less than 5 mU/l 30 minutes after an i.v. bolus of TRH was seen in 15 patients in group I, in none of the patients in group II.

Compound S levels less than 10 μg/dl were observed in 3 of 15 patients in group I, but in none of group II. The mean value of Compound S in group I (15.1 $\pm$ 1.8 μg/dl) was significantly lower than in group II (22.8 $\pm$ 2.7 μg/dl).

Spermanalyses of both groups are given in Table 3.3. These analyses could be assessed in only 4 patients of group I, who were able to ejaculate. All parameters were normal with the exception of decreased motility in all 4 specimens. One of these patients became father several weeks before admission to our hospital. Two patients of group II (patients 1 and 12) fathered wihout any therapy several months after diagnosis. The wives of 4 men treated with bromocriptine conceived during therapy as shown in Table 3.4. A small but significant ($P<0.05$) rise of the serum testosterone level was present in all these 4 patients. Another 6 patients were treated with bromocriptine; PRL levels normalized but conception was not achieved. In 1 patient bromocriptine could not be administered because of adverse side effects.

TABLE 3.3 Sperm qualities of 13 untreated hyperprolactinemic men referred because semen abnormalities were suggested to be the cause of their infertility and of 4 men with PRL-secreting macroadenomas (a-d).

patient	volume ml	count $x10^6$/ml	motility %	normal spermatozoa %
1	6	1.2	0	48
2	4	25	25	15
3	2	2	10	5
4[a]	4	<1	0	24
5[a]	2	10	5	21
6	3	15	35	26
7	5	<1	30	2
8	8	11	30	18
9	3	<1	0	1
10	5	10	10	21
11	5	90	10	34
12	3	60	10	41
13	8	7	10	9
a	2	138	20	6
b	3	30	30	23
c	1.5	275	25	21
d	3.5	30	15	8

[a] macroadenomas

Table 3.4 RESPONSE OF PRL, LH, FSH, TESTOSTERONE AND SPERM QUALITIES TO BROMOCRIPTINE THERAPY IN 4 INFERTILE MEN WHOSE WIVES CONCEIVED

months	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
patient 2																														
sperm volume (ml)	4								2		2		4		3				3						1					
count (10^6/ml)	25								25		30		10		13				15						9					
motility (%)	25								30		40		20		30				50						55					
normal spermatozoa (%)	15								41		57		16		8				10						43					
PRL (µg/l)	61				84				4			6		6					2				5						
FSH (U/l)	2.2				2.8				2.9			2.3		2.6					2.8				2.5						
LH (U/l)	1.3				1.2				3.2			3.5		1.5					2.7				2.9						
T (µg/dl)	.26				.28				.61			.65		.68					.72				.74						
bromocriptine																	●				▲									
patient 4																														
sperm volume (ml)	4				7			5			6							6			6			6					5	
count (10^6/ml)	1				1			4			3							4			4			6					10	
motility (%)	0				0			5			45							35			25			20					50	
normal spermatozoa (%)	24				19			26			34							16			12			6					10	
PRL (µg/l)	43				13						19							24			30			12					9	
FSH (U/l)	11.5				11.7						13.3							12.4			8.8			12.4					13.4	
LH (U/l)	2.6				1.9						3.9							4.2			3.0			4.0					4.3	
T (µg/dl)	.30				.46													.33			.33			.60					.52	
bromocriptine			●						▲																				▲	
patient 7																														
sperm volume (ml)	5			5				4						5	5															
count (10^6/ml)	1			1				1						2	1															
motility (%)	30			50				0						0	0															
normal spermatozoa (%)	2			10				5																						
PRL (µg/l)	23		2					1				9			12															
FSH (U/l)	10.4		9.8					9.5				10.7			6.7															
LH (U/l)	3.9		4.4					5.6				5.6			3.8															
T (µg/dl)	.36		.27					.52				.35			.33															
bromocriptine												▲																		
patient 8																														
sperm volume (ml)		8		8				7											8			8								
count (10^6/ml)		11		20				50											70			32								
motility (%)		30		20				60											75			65								
normal spermatozoa (%)		18		29				13											23			35								
PRL (µg/l)	25									59					27	8		10			8						8		
FSH (U/l)	0.9														1.1	1.4												0.6		
LH (U/l)	1.5														2.9	1.8												2.4		
T (µg/dl)	.44														.47	.54												.60		
bromocriptine							●									▲														

● = ligation of spermatical vein, ▲ = conception.

4. Discussion

In the present study impotence and loss of libido was especially associated with a PRL-secreting macroadenoma of the pituitary and high PRL levels The majority of these patients, however, was referred with symptoms of visual impairment, headache or hypopituitarism. Both impotence and loss of libido were absent in 11 infertile patients of group II displaying only moderately increased PRL levels. In this group of patients the diagnosis PRL-secreting microadenoma was made by excluding other causes of hyperprolactinemia. Two patients, who experienced decreased libido showed an enlarged sella turcica. Previous reports have indicated that impotence, loss of libido and hypogonadism are considered to be common clinical features of hyperprolactinemia in men with PRL-secreting pituitary adenomas, but that the presenting symptoms are often related to local expansion of the tumor (Carter et al., 1978; Franks et al., 1978; Nagulesparen et al., 1978; Thorner & Besser, 1978; Derome et al., 1979; Grisoli et al., 1980; Serri et al., 1980; Spark et al., 1982). In our study testosterone levels were decreased in the patients with macroadenomas (group I), but mostly normal in group II, which might suggest that only high levels of PRL are accompanied by low levels of testosterone. This is in contrast with a recent report by Spark et al. (1982), who described decreased serum testosterone levels in men with microadenomas and slightly increased PRL levels.

The mechanism(s) by which sexual dysfunction and hypogonadism develop in hyperprolactinemic men are unclear. Low testosterone levels imply either deficient gonadotropin secretion or inhibition of the go-

nadotropins at the gonadal level by PRL. In our study basal levels of serum LH and FSH in hyperprolactinemic men did not differ from the control group, which is in accordance with other reports (Carter et al., 1978; Peillon et al., 1979). The basal levels of both LH and FSH were significantly increased in the group of infertile patients with low sperm counts compared to group I. This observation is in accordance with a previous report that sperm count is negatively correlated with FSH and LH (Aafjes et al., 1977). Nevertheless, a suppressive effect on basal gonadotropins in patients with macroadenomas and high PRL levels (group I) cannot be denied. A blunted response of LH and FSH however was present in group I. The response of LH and FSH in group II and the control group were not different. Similar results have been observed by others (Nagulesparen et al., 1978; Eversmann et al., 1981). Eversmann et al. (1981) showed that 90% of his patients with PRL-secreting macroadenomas had an insufficient response of LH and FSH to LRH. Other studies however report normal LRH tests in men with macroadenomas (Franks et al., 1978: Prescott et al., 1982). No possible explanation can be given for these contradictory results. Based on the discussion above, it seems likely that reduced levels of testosterone are due to impaired gonadotropin secretion. However, there is also some evidence for a direct effect of PRL on the level of the gonads. A fairly quick increase of serum testosterone levels can be observed during bromocriptine therapy despite little or no increase of serum LH (Carter et al., 1978). Moreover a direct effect of PRL not only on Leydig cell function but als on the accessory sex glands has been suggested by Segal et al. (1979) in infertile hyperprolac-

tinemic men displaying small prostates and decreased seminal plasma volume. Normal Leydig cell function has been reported by others (Carter et al., 1978; Franks et al., 1978) based on a normal increase of testosterone after stimulation with hCG in hyperprolactinemic men. Although testosterone levels were mostly normal in our infertile patients we had the opportunity to study the effects of normalization of serum PRL levels in these hyperprolactinemic men with normal anterior pituitary function. Moreover, the effects of normalizing PRL levels on sexual disturbances could be studied. It has been shown that impotence does not improve after normalization of serum testosterone levels in the presence of elevated PRL levels (Carter et al., 1978; Luboshitzky et al., 1979), whereas improvement of potency was noted after normalization of serum PRL levels in spite of continued low testosterone levels (Nagulesparen et al., 1978).

Sperm analyses of the 4 men in group I with macroadenomas were normal with the exception of decreased motility. One of these patients fertilized several weeks before admission to our hospital. Moreover 2 patients in group II with microadenomas were able to father children after the time of diagnosis of their hyperprolactinemia without any treatment. These findings suggest that hyperprolactinemia might not be responsible for the sperm abnormalities in these patients. Otherwise the 4 men who were treated with bromocriptine and fertilized showed a small however significant increase of serum testosterone levels. The fact that basal and post-LRH levels of serum gonadotropins were normal in these patients and did not change during treatment suggests that originally not only the testosterone secretion was impaired but also

the feed-back of testosterone on LH secretion. Sperm analyses of men with prolactinomas may reveal azoospermia, oligozoospermia, normozospermia and even polyzoospermia (Snyder et al., 1979). However, reports on fertility are very sparse. A more valid observation was done by Jequier et al. (1979) who described normal spermatogenesis in testicular biopsies from hyperprolactinemic men. Sperm qualities did not improve dramatically in our patients who fertilized during treatment. The increase of testosterone might lead to an improvement of the function of the accessory sex glands. However, 3 of these 4 patients had a varicocele and underwent ligation of the spermatical vein: this might have caused fertility (Dubin et al., 1975). Recently it has been shown that conception was achieved in 3 hyperprolactinemic men with long standing infertility during treatment with bromocriptine (Laufer et al., 1981). The increased sperm motility in this study underlines the possibility of improved accessory sex gland function. Thus it is possible that PRL has a direct effect.

Finally, the immediate improvement of diminished libido in 2 infertile patients after normalization of serum PRL levels might suggest a direct effect of PRL on the brain as well.

In conclusion our data suggest that impotence and loss of libido in hyperprolactinemic men are related to the presence of macroadenomas and high PRL levels. We consider that compressive effects of the tumor account for the impairment of gonadotropin secretion and other anterior pituitary functions. The impairment of testosterone secretion could be explained by a defective pituitary-gonadal axis in these patients. However, a direct effect of PRL on the brain, pituitary and on the gonads could not be ruled out.

Decreased libido and testosterone levels in the lower normal range improved after normalization of serum PRL levels with bromocriptine. Fertilization during bromocriptine therapy also coincided very well with a rise in testosterone levels, without any change in sperm qualities.

5. Summary

Thirty-two men with untreated prolactinomas were studied. Nineteen patients (group I), referred because of headache, visual impairment or hypopituitarism, had a macroadenoma and high PRL levels (982 $\pm$ 294 μg/l; mean $\pm$ S.E.M.). Impotence was present in 12, loss of libido in 9 patients. Eleven patients of group II, comprising 13 patients out of a group of 598 infertile men, had a microadenoma (PRL levels: 42 $\pm$ 8 μg/l).

Anterior pituitary functions, evaluated by the basal levels of LH, FSH, TSH and the consecutive administration of LRH and TRH were normal in all patients of group II. Blunted responses, subnormal levels of testosterone, T_4 and Compound S (after metyrapone) were especially present in group I, which is considered to be due to compressive effects of the tumor.

The wives of 4 of 11 patients in group II treated with bromocriptine conceived. 2 Patients in group II and 1 in group I fathered without any treatment, indicating that infertility is not necessarily a consequence of hyperprolactinemia.

Improvement of libido and increase of testosterone levels during bromocriptine treatment may suggest a direct effect of PRL.

6. References

Aafjes, J.H., van der Vijver, J.C.M., Docter, R. & P.E. Schenck. Serum gonadotrophins, testosterone and spermatogenesis in subfertile men. Acta Endocrinol. 1977, 86, 651-658.

Åbyholm, T. & K. Molne. Serum prolactin and radiological skull examinations in infertile men with azoospermia or severe oligozoospermia. Int. J. Androl. 1980, 3, 229-235.

Carter, J.N., Tyson, J.E., Tolis, G., van Vliet, S., Faiman, C. & H.G. Friesen. Prolactin-secreting tumors and hypogonadism in 22 men. N. Engl. J. Med. 1978, 299, 847-852.

Derome, P.J., Peillon, F., Bard, R.H., Jedynak, C.P., Racadot, J. & G. Guiot. Adénomes à prolactine: résultats du traitement chirurgical. Nouv. Presse Med. 1979, 8, 577-583.

Dubin, L. & R.D. Amelar. Varicocelectomy as therapy in male infertility: a study of 504 cases. Fertil. Steril. 1975, 26, 217-220.

Eversmann, T., Eichinger, R., Fahlbusch, R., Rjosk, H.K. & K. von Werder. Die Hyperprolaktinaemie beim Mann: Klinik und Therapie. Schweiz. Med. Wochenschr. 1981, 47, 1782-1789.

Franks, S., Jacobs, H.S., Martin, N & J.D.N. Nabarro. Hyperprolactinaemia and impotence. Clin. Endocrinol. 1978, 8, 277-287.

Grisoli, F., Vincentelli, F., Jaquet, P., Guibout, M., Hassoun, J. & P. Farnarier. Prolactin secreting adenoma in 22 men. Surg. Neurol. 1980, 13, 241-247.

Hargreave, T.B., Kyle, K.F., Kelly, A.M. & P. England. Prolactin and gonadotrophins in 208 men presenting with infertility. Br. J. Urol. 1977, 47, 747-750.

Hermanns, U & E.S.E Hafez. Prolactin and male reproduction. Arch. Androl. 1981, 6, 95-125.

Jequier, A.M , Crich, J.C. & I D. Ansell. Clinical findings and testicular histology in three hyperprolactinemic infertile men Fertil. Steril. 1979, 31, 525-530.

Klijn, J.G.M., Lamberts, S.W.J., de Jong, F.H., Docter, R., van Dongen, K.J. & J.C Birkenhäger. The importance of pituitary tumour size in patients with hyperprolactinaemia in relation to hormonal variables and extrasellar extension of tumour. Clin. Endocrinol. 1980, 12, 341-355.

Klijn, J.G.M., Lamberts, S.W.J., de Jong, F.H. & J.C. Birkenhäger. The value of the TRH-test in patients with prolactin secreting pituitary tumors and supra-sellar non-pituitary tumors. Fertil. Steril. 1981, 35, 155-161.

Laufer, N , Yaffe, H., Margalioth, E.J., Livshin, J., Ben-David, M. & J.G. Schenker. Effect of bromocriptine treatment on male infertility associated with hyperprolactinemia. Arch. Androl. 1981, 6, 343-346.

Luboshitzky, R., Rosen, E., Trestian, S. & I.M. Spitz. Hyperprolactinaemia and hypogonadism in men: response to exogenous gonadotrophins. Clin. Endocrinol. 1979, 11, 217-223.

Meikle, A.W., Jubiz, W., Hutchings, M.P., West, C.D. & F.H. Tyler. A simplified metyrapone test with determination of plasma 11-deoxycortisol (metyrapone test with plasma S). J. Clin. Endocrinol. Metab. 1969, 29, 985-987.

Miller, J.B., Howards, S S. & R.M. Macleod. Serum prolactin in organic and psychogenic impotence. J. Urol. 1980, 123, 862-864.

Nagulesparen, A., Ang, V. & J.S. Jenkins. Bromocriptine treatment of males with pituitary tumours, hyperprolactinaemia, and hypogonadism. Clin. Endocrinol. 1978, 9, 73-79.

Peillon, F., Bard, H., Mowszowicz, I., Cesselin, F., Lagoguey, M. & F. Boyet. Les adénomes à prolactine chez l'homme. Ann. Endocrinol. (Paris) 1979, 40, 73-74.

Perryman, R.L. & M.O. Thorner. The effects of hyperprolactinemia on sexual and reproductive function in men. J. Androl. 1981, 5, 233-242.

Prescott, R.W.G., Johnston, D.G., Kendall-Taylor, P., Crombie, A., Hall, K., McGregor, A. & R. Hall. Hyperprolactinaemia in men - response to bromocriptine therapy. Lancet 1982, 1, 245-248.

Segal, S., Yaffe, H., Laufer, N. & M. Ben-David. Male hyperprolactinemia: effects on fertility. Fertil. Steril. 1979, 32, 556-561.

Serri, O., Somma, M , Rasio, E., Beauregard, H. & J. Hardy. Prolactin-secreting pituitary adenomas in males: transsphenoidal microsurgical treatment. Can. Med. Assoc. J. 1980, 122, 1007-1013.

Snyder, P.J., Bigdeli, H., Gardner, D F., Mihailovic, V., Rudenstein, R.S., Sterling, F.H. & R.D. Utiger. Gonadal function in fifty men with untreated pituitary adenomas. J. Clin. Endocrinol. Metab. 1979, 48, 309-314.

Spark, R.F., White, R A. & P.B. Connolly. Impotence is not always psychogenic. JAMA 1980, 243, 750-755.

Spark, R.F., O'Reilly, G., Wills, C.A., Ransil, B.J. & R. Bergland. Hyperprolactinaemia in males with and without pituitary macroadenomas. Lancet 1982, 2, 129-131.

Thorner, M O. & G.M. Besser. Bromocriptine treatment of hyperprolactinaemic hypogonadism. Acta Endocrinol. 1978, 88, Suppl. 216, 131-146.

Verjans, H.L., Cooke, B.A., de Jong, F.H., de Jong, C.M.M. & H.J. van der Molen. Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid. Biochem. 1973, 4, 665-676.

Visser, T.J., van den Hout-Goemaat, N.L., Docter, R. & G. Hennemann. Radioimmunoassay of thyroxine in unextracted serum. Neth. J. Med. 1975, 18, 111-115.

J. ENDOCRINOL. 1982, 93, 223-229

CHAPTER 4

EFFECTS OF A PRL-SECRETING TUMOR ON COPULATORY BEHAVIOR IN MALE RATS

R.F.A. Weber, M.P. Ooms, J.T.M. Vreeburg

1. Introduction

Increased levels of serum PRL in men with a pituitary tumor have been associated with a loss of libido and potency (Carter et al., 1978; Franks et al., 1978; Thorner & Besser, 1978). Since suppression of hyperprolactinemia in such patients by treatment with bromocriptine frequently results in restoration of libido and potency, it has been suggested that hyperprolactinemia causes a decline in libido and potency (Carter et al., 1978; Franks et al., 1978; Thorner & Besser, 1978).

Our knowledge concerning the effects of PRL on mating behavior of experimental animals is fragmentary. In rats increased levels of PRL can be induced by grafting pituitary glands under the kidney capsule. Recently, it has been demonstrated that 5 days after pituitary grafting several parameters of mating behavior in male rats were enhanced. In particular, significant reductions in the mount and intromission latencies were observed (Drago et al., 1981). In long-term experiments, however, mating behavior of such pituitary-grafted animals appeared to be suppressed; the rats exhibiting increased latency to mount and intromission and reduced frequency of in-

tromission (Svare et al., 1979). In addition, few rats with hyperprolactinemia ejaculated. Since a sustained increase of plasma PRL did not affect plasma testosterone in these rats it may be argued that the effects of hyperprolactinemia on mating behavior are brought about by a direct action on the brain (Bartke, 1980). Indeed, there is strong evidence that PRL has a direct action on the brain; PRL enhances dopamine turnover in the hypothalamus (Gudelsky et al., 1976) and stimulates the secretion of dopamine into hypophysial portal blood (Cramer et al., 1979).

In rats bearing transplantable PRL-producing tumors, the serum concentration of PRL reaches levels of several μg NIAMDD-PRL RP-1/ml (Cramer et al., 1979; Lamberts & MacLeod, 1979; Hodson et al., 1980; Panerai et al., 1980), levels which are much higher than those found in pituitary-grafted rats. The present study was carried out to investigate the mating behavior of rats bearing tumor 7315a, a tumor which secretes PRL and adrenocorticotropin (ACTH) (MacLeod et al., 1968). Since the presence of this tumor is accompanied in female rats by suppressed plasma levels of LH and FSH (Lamberts et al., 1981), the rats were castrated and testosterone-filled silicone elastomer capsules were implanted in order to prevent differences in testosterone concentrations between tumor-bearing and control animals. In an additional experiment, rats were adrenalectomized to evaluate the effects of the tumor-stimulated adrenals on male copulatory behavior.

2. Materials and methods

Male rats of the Buffalo strain were kept in a

room where the lights were on between 19.00 and 09.00 h. Beginning 2 weeks after arrival in our laboratory, each male was placed with a receptive female for at least 30 min in a plastic cage identical to his home cage to obtain sexual experience. These females were ovariectomized and made sexually receptive by s.c. injections of 10 μg oestradiol benzoate 48 h before testing and 1 mg progesterone 4 h before testing. This procedure was carried out twice weekly for 3 weeks. All animals ejaculated during the last three tests. Subsequently, the rats were castrated (experiment 1) and received a 1 cm long silicone elastomer capsule (Talas, Zwolle, The Netherlands; outside diameter 0.1 cm) filled with testosterone (Steraloids Inc., Wilton, New Hampshire, U.S.A.). In experiment 2, castrated rats were adrenalectomized. Adrenalectomized rats received 0.9% NaCl (w/v) solution for drinking. On the morning of day 4 after surgery the animals in experiment 1 were injected s.c. in the neck region with 0.2 ml tumor suspension. The tumor suspension was prepared by mincing tumor tissues with twice its volume of 0.9% NaCl solution. Test 1 for male copulatory behavior was carried out during the afternoon of this day. Behavioral test 2 took place on day 7 after tumor inoculation, test 3 on day 14, test 4 on day 21, test 5 on day 28 and test 6 on day 35. The rats in experiment 2 were injected with tumor suspension on the morning of day 6 after castration and adrenalectomy. Test 1 was performed during the afternoon of this day. Behavioral test 2 was carried out on day 7 after tumor inoculation, test 3 on day 14, test 4 on day 21, test 5 on day 28, test 6 on day 35 and test 7 on day 42. All tests were carried out in a dimly lit room.

2.1. Behavioral testing

After adaptation of the male to the test cage for 5 min, a receptive female was introduced and male copulatory behavior was scored. After introduction of the female, males were given 15 min to achieve an initial intromission, and if this occurred an additional 15 min to ejaculate. In the event of an ejaculation, males were left in the test cage until the first intromission of a second copulatory series occurred. The following parameters of masculine behavior were scored: (a) contact latency: the time which elapsed between the introduction of the female and the first mount (with pelvic thrusting) or intromission; (b) ejaculation latency: the time which elapsed from the first intromission until ejaculation; (c) the number of mounts preceding ejaculation; (d) the number of intromissions preceeding ejaculation; (e) post-ejaculatory interval: the time which elapsed between ejaculation and the first subsequent mount or intromission.

For each animal, the mean value was calculated of a specified parameter scored in test 1 and 2 (normal plasma PRL, see below), tests 3 and 4 (moderately increased plasma PRL) and in the last two or three tests (markedly increased plasma PRL). From these individual means, group means and standard errors were determined. When an animal did not show a specified behavior in both tests, the score obtained in only one test was used.

2.2. Statistical analysis

Results are presented as means $\pm$ S.E.M. The data

were subjected to split-plot 2.3 design analysis of variance, the unweighted means solution was used because of unequal numbers of rats (Kirk, 1968) and $P<0.05$ was adopted as the level of statistical significance. Significant interactions were tested with simple main effects. With significant F ratios, the differences between the means were tested with the t-ratio procedure (Kirk, 1968).

2.3. Hormone determinations

Four times during experiment 1 (Fig. 4.1a) and three times during experiment 2 (Fig. 4.1b) blood was taken under ether anesthesia. Serum PRL was determined by a double-antibody radioimmunoassay using materials and protocols supplied by the NIAMDD. The

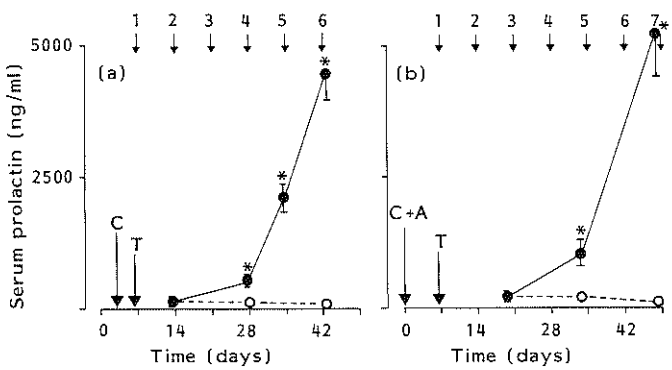

Fig. 4.1 Serum levels of prolactin (means ± S.E.M.) in (a) castrated and (b) castrated and adrenalectomized tumour-bearing (solid lines) or control (broken lines) rats. All rats were implanted with replacement testosterone. Time of castration (C), adrenalectomy (A) and tumour inoculation (T) are shown and the arrows at the top indicate when the tests were carried out. $*P < 0.01$ compared with control rats (analysis of variance).

interassay coefficient of variation was 15%. The results are expressed in terms of NIAMDD-rat-PRL RP-1. Testosterone concentrations were measured by radioimmunoassay, using the method and the antiserum first described by Verjans et al. (1973). The interassay and intra-assay coefficients of variation were 15 and 7% respectively.

3. Results

3.1. Serum levels of PRL and testosterone

Serum PRL concentrations of castrated tumor-bearing rats are shown in Figure 4.1a. As may be seen, 6 days after tumor inoculation the PRL levels measured in the tumor-bearing rats were similar to those found in control animals. However, serum samples collected from tumor-bearing rats 20, 27 and 34 days after tumor inoculation contained significantly higher PRL levels than those found in control rats. Similar observations were made in the tumor-bearing rats after castration and adrenalectomy (Figure 4.1b). Twelve days after tumor inoculation serum PRL levels were as high in the control group as in the tumor-bearing group. The serum PRL levels measured in the tumor-bearing rats 26 and 40 days after tumor inoculation were significantly higher in the tumor-bearing rats than in the control animals.

After castration, testosterone was administered using silicone elastomer capsules filled with testosterone. In tumor-bearing castrated rats serum testosterone levels were similar to those found in castrated control animals. Testosterone concentrations 6 and 27 days after tumor inoculation in tumor-bear-

ing rats were estimated to be 0.69 ± 0.05 and 0.46 ± 0.11 ng/ml respectively. In control animals 0.49 ± 0.01 and 0.63 ± 0.07 ng testosterone/ml was measured. In castrated and adrenalectomized rats on days 12 and 40 after tumor inoculation the testosterone levels were 0.79 ± 0.06 and 0.51 ± 0.03 ng/ml. In these castrated or castrated and adrenalectomized rats the testosterone-filled capsules induced serum testosterone concentrations which were much lower than those found recently in our laboratory in groups of intact (2.8 ± 0.2 ng/ml) or adrenalectomized (3.2 ± 0.5 ng/ml) rats of the same age and strain.

3.2. Copulatory behavior

Parameters of male copulatory behavior in castrated tumor-bearing rats and control animals are shown in Table 4.1. Analysis of variance revealed that none of the parameters for male sexual behavior in control rats changed during the period of testing. However, in tumor-bearing rats a signifcant ($P<0.01$) increase in contact latency and ejaculation latency was found. In tests 3+4 the mean ejaculation latency of tumor-bearing rats was significantly longer than that found in control animals. The mean contact and ejaculation latencies, measured in tests 5+6 were significantly longer than those observed in control animals. Although no change in the number of intromissions was found, the mean number of mounts before ejculation increased significantly ($P<0.01$) in the course of the experiment. The number of mounts displayed by tumor-bearing rats in tests 3+4 and tests 5+6 were significantly higher than those found in control rats. The rise in the number of mounts ob-

served in tumor-bearing rats was not only reflected in the increased ejaculation latency but also in the reduced proportion of animals ejaculating in tests 5+6, although this reduction did not reach significance.

After adrenalectomy and castration significant changes in the parameters of male copulatory behavior did not develop in either the tumor-bearing rats or the controls (Table 4.1).

4. Discussion

In the present study an increase in serum PRL levels was induced by the transplantable tumor 7315a. Since the serum levels of PRL measured within 12 days after tumor inoculation were not significantly raised, the first and second tests for male copulatory behavior were carried out when serum PRL levels were normal. However, serum PRL increased sharply during the course of the experiment. Two days before test 5 serum PRL concentrations of more than 2000 ng/ml in castrated and more than 1000 ng/ml in castrated and adrenalectomized rats were found. At the time of the last tests, more than 4000 ng PRL/ml serum was present. The extremely high levels of PRL in rats bearing tumor 7315a have also been found by other investigators (Cramer et al., 1979; Lamberts & MacLeod, 1979; Panerai et al., 1980).

The data we have presented have shown that in castrated rats implanted with testosterone-filled capsules, tumor 7315a has a markedly inhibitory effect on male copulatory behavior. In the course of the experiment tumor-bearing rats displayed a growing

TABLE 4.1 Copulatory behavior of castrated and of castrated and adrenalectomized rats after administration of testosterone and tumor inoculation.

test	group	rats ejaculating twice †	no. of mounts before ejaculation	no. of intromissions before ejaculation	contact latency (s)	ejaculatory latency (s)	post-ejaculatory interval (s)
castrated rats							
1+2	tumor	7/7	5.2 ± 1.7	14.7 ± 2.2	21.7 ± 11.2	287 ± 43	218 ± 9
	control	6/6	5.8 ± 1.8	13.8 ± 1.0	14.8 ± 5.1	327 ± 47	247 ± 15
3+4	tumor	7/7	24.1 ± 5.7^{x}	14.7 ± 1.5	41.6 ± 25.0	493 ± 72^{x}	230 ± 11
	control	6/6	6.5 ± 0.9	12.8 ± 0.8	15.7 ± 8.9	286 ± 30	242 ± 10
5+6	tumor	4/7	52.3 ± 12.2^{xx}	11.7 ± 1.1	325.1 ± 97^{xx}	836 ± 130^{xx}	273 ± 34
	control	6/6	7.5 ± 1.1	14.5 ± 1.3	20.5 ± 14.9	296 ± 29	212 ± 6
castrated and adrenalectomized rats							
1+2	tumor	5/7	18.9 ± 5.0	11.4 ± 0.8	82 ± 38	613 ± 125	340 ± 16
	control	6/7	15.8 ± 3.1	11.0 ± 0.7	68 ± 15	516 ± 73	325 ± 10
3+4	tumor	5/7	17.9 ± 5.9	9.6 ± 0.7	47 ± 16	512 ± 113	366 ± 36
	control	5/7	8.4 ± 2.2	10.1 ± 0.8	187 ± 104	422 ± 53	403 ± 62
5+6+7	tumor	5/7	29.0 ± 5.7	11.1 ± 0.9	84 ± 20	892 ± 153	378 ± 37
	control	7/7	18.4 ± 3.4	9.5 ± 0.6	175 ± 37	576 ± 82	342 ± 61

values are means ± S.E.M.

^{x} $P<0.05$, ^{xx} $P<0.01$ compared with control rats (analysis of variance)

† all animals ejaculated at least once

number of mounts before ejaculation. The number of intromissions leading to ejaculation, however, remained the same. The increase in ejaculation latency seems therefore to be the consequence of a relative inability to intromit. Although (even during the last tests) tumor-bearing animals were sexually very active, mounting or intromitting as frequently as control rats, their contact latency was significantly increased. Our findings are similar in part to those found by Svare et al., (1979). These investigators, studying copulatory behavior of pituitary-grafted male rats, also found increase mount and ejaculation latencies. In contrast to our findings, they reported a decrease in the proportion of rats which ejaculated and a reduction in the number of mounts and intromissions.

The differences in mating behavior between tumor-bearing rats and control rats were no longer present when the adrenals were removed before tumor inoculation. This observation suggests that secretory products of the adrenal are involved in the suppression of mating behavior. Tumor 7315a secretes both PRL and ACTH (MacLeod et al., 1968) resulting in adrenals which at autopsy were five times heavier in the tumor-bearing rats than in the control rats (data shown in Chapter 5). It is unclear therefore whether PRL, ACTH or a combination of both hormones are needed to cause the inhibitory action of the adrenal.

An alternative explanation for the abscence of an effect of PRL on mating behavior after adrenalectomy might be that this hormone exerts its inhibitory effect only in the presence of corticosteroids. Doherty et al., (1980) recently studied copulatory behavior of pituitary-grafted adrenalectomized rats. They reported that pituitary grafts prolonged the la-

tencies to mount and intromit both in intact and adrenalectomized rats and concluded that the suppression of copulatory behavior is not due to stimulation of the adrenal activity by hyperprolactinemia. The discrepancy between our results and those of Doherty et al. (1980) might be caused by the fact that the latter investigators injected the adrenalectomized rats with corticosterone. An interaction between PRL and corticosteroids is well known as a cause of milk production and was confirmed in the present experiments. In tumor-bearing male rats mammary glands full of milk were found, whereas in adrenalectomized tumor-bearing animals the glands were empty. In addition, a synergism between PRL and the adrenals seems to be involved in the suppression of LH during hyperprolactinemia (Greely & Kizer, 1979).

Hyperprolactinemia in men is associated both with hypogonadism and impotence (Carter et al., 1978; Franks et al., 1979; Thorner & Besser, 1978; Kirby et al., 1979). Therefore, the impotence might be related to increased serum PRL in combination with low testosterone. We therefore supplied the rats after castration with relatively small testosterone-filled capsules. Testosterone concentrations were similar in tumor-bearing and control rats, showing that PRL has no significant influence on the metabolic clearance rate of testosterone.

It has been shown recently (Panerai et al., 1980) that besides PRL and ACTH, tumor 7315a secretes β-endorphin. This opioid peptide has been found to suppress copulatory behavior in male rats after intraventricular injection (Meyerson et al., 1977; McIntosh et al., 1980). Inhibition of male copulatory behavior has also been reported after systemic administration of 4-10 ACTH to castrated rats which

were injected with testosterone propionate (Bohus et al., 1975). Since, if anything, these peptides inhibit male copulatory behavior, the conclusion that PRL does not suppress copulatory behavior in adrenalectomized rats is valid. Whether this is the case in adrenalectomized rats injected with corticosterone will be investigated.

5. Summary

The effect of the transplantable PRL- and ACTH-secreting tumor 7315a on male copulatory behavior was investigated. Castrated tumor-bearing rats implanted with testosterone-filled capsules exhibited significantly longer latencies to first mount or intromission and to ejaculation than castrated and substituted control animals. In contrast to the number of intromissions, the number of mounts before ejaculation of the tumor-bearing rats was considerably increased. However, when castration was carried out in addition to adrenalectomy, the differences in copulatory behavior between tumor-bearing rats and control rats were no longer present. During the last tests for copulatory behavior the tumor-bearing rats had serum PRL concentrations of more than 4000 ng/ml while control rats had less than 100 ng/ml. Plasma testosterone levels produced by silicone elastomer capsules were neither affected by the presence of the tumor nor by adrenalectomy. It was concluded that hyperprolactinemia does not suppress the copulatory behavior of adrenalectomized rats.

6. References

Bartke, A. Role of prolactin in reproduction in male mammals. Fed. Proc. 1980, 39, 2577-2581.

Bohus, B., Hendrickx, H.H.L., van Kolfschoten, A.A. & T.G. Krediet. Effect of ACTH 4-10 on copulatory and sexually motivated approach behavior in the male rat. In: Sexual behavior: pharmacology and biochemistry. Eds. M. Sandler & G.L. Gessa. Raven Press N.Y. 1975, 269-275.

Carter, J.N., Tyson, J.E., Tolis, G., van Vliet, S., Faiman, C. & H.G. Friesen. Prolactin-secreting tumors and hypogonadism in 22 men. N. Engl. J. Med. 1978, 299, 847-852.

Cramer, O.M., Parker, C.R. & J.C. Porter. Secretion of dopamine into hypophyseal portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinology 1979, 105, 636-640.

Doherty, P.C., Smith, M.S. & A. Bartke. Hyperprolactinemia and reproductive function in male rats. Effects of adrenalectomy and bromocriptine. Program of the U.S. Endocrine Society, 62nd Annual Meeting. Abstract No. 704.

Drago, F., Pellegrini-Quarantotti, B., Scapagnini, U. & G.L. Gessa. Short-term endogenous hyperprolactinemia and sexual behavior of male rats. Phys. Behav. 1981, 26, 277-279.

Franks, S., Jacobs, H.S., Martin, N. & J.D.N. Nabarro. Hyperprolactinaemia and impotence. Clin. Endocrinol. 1978, 8, 277-287.

Greeley, G.H. & J.S. Kizer. Evidence for adrenal involvement in the modulatory role of prolactin in luteinizing hormone secretion in the male rat. Endocrinology 1979, 104, 948-953.

Gudelsky, G.A., Simpkins, J., Mueller, G.P., Meites, J. & K.E. Moore. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 1976, 22, 206-215.

Hodson, C.A., Simpkins, J.W., Pass, K.A., Aylsworth, C.F., Steger, R.W. & J. Meites. Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinology 1980, 30, 7-10.

Kirby, R.W., Kotchen, T.A. & E. Douglas Rees. Hyperprolactinemia - A review of recent clinical advances. Arch. Int. Med. 1979, 139, 1415-1419.

Kirk, R.E. Experimental design: procedures for the behavioral sciences. Brooks/Cole, 1968. Belmont, California.

Lamberts, S W.J. & R.M. MacLeod. The inability of bromocriptine to inhibit prolactin secretion by transplantable rat pituitary tumors: observations on the mechanism and dynamics of the autofeedback regulation of prolactin secretion. Endocrinology 1979, 104, 65-70.

Lamberts, S.W J., Zuiderwijk, J.M., Bons, E.G., Uitterlinden, P. & F.H. de Jong. Gonadotropin secretion in rats bearing a prolactin-secreting pituitary tumor: effects of naloxone administration. Fertil. Steril. 1981, 35, 557-562.

McIntosh, T.K., Vallano, M.L. & R.J. Barfield. Effects of morphine, β-endorphin and naloxone on catecholamine levels and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 1980, 13, 435-441.

MacLeod, R.M., DeWitt, G.W. & M C. Smith. Suppression of pituitary gland hormone content by pituitary tumor hormones. Endocrinology 1968, 82, 889-894.

Meyerson, B.J. & L. Terenius. β-Endorphin and male sexual behavior. Eur. J. Pharmacol. 1977, 42, 191-192.

Panerai, A.E., Sawynok, J., LaBella, F.S. & H.G. Friesen. Prolonged hyperprolactinemia influences β-endorphin and met-enkephalin in the brain. Endocrinology 1980, 106, 1804-1808.

Svare, B., Bartke, A., Doherty, P., Mason, I., Michael, S.D. & M.S. Smith. Hyperprolactinemia suppresses copulatory behavior in male rats and mice. Biol. Reprod. 1979, 21, 529-535.

Thorner, M.O. & G.M Besser. Bromocriptine treatment of hyperprolactinaemic hypogonadism. Acta Endocrinol. 1978, 88, Suppl. 216, 131-146.

Verjans, H.L., Cooke, B.A., de Jong, F.H., de Jong, C.M.M. & H J. van der Molen. Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid. Biochem. 1973, 4, 665-676.

ENDOCRINOLOGY 182, 111, 412-417

CHAPTER 5

EFFECTS OF A PRL- AND ACTH-SECRETING TUMOR ON GONADOTROPIN LEVELS AND ACCESSORY SEX ORGAN WEIGHTS IN ADULT MALE RATS: A POSSIBLE ROLE OF THE ADRENALS

R.F.A. Weber, M.P. Ooms and J.T.M. Vreeburg

1. Introduction

It is well established that chronically elevated plasma PRL levels induced by pituitary grafts suppress basal gonadotropin concentrations in intact male rats (Bartke et al., 1977; Celotti et al., 1979; McNeilly et al., 1978; Greeley & Kizer, 1979). Since plasma testosterone levels in these animals were not affected (Bartke et al., 1977; McNeilly et al., 1978), it seems reasonable to conclude that high levels of PRL exert an inhibitory effect on gonadotropin secretion. This inhibitory effect of PRL on gonadotropin secretion in intact male rats is even more pronounced after the implantation of a PRL-producing tumor (MtTW15); the extremely high plasma PRL levels induced by this tumor are accompanied by a strong suppression not only of serum LH but also of serum testosterone (Hodson et al., 1980). Besides the suppression of basal gonadotropin secretion in intact animals, PRL can also inhibit the rise of plasma gonadotropins after gonadectomy (Gudelsky et al., 1976; Winters & Loriaux, 1978; McNeilly et al., 1980). As in intact animals, the inhibition of gonadotropin secretion in gonadectom-

ized rats treated with PRL-producing tumors (Grandison et al., 1977; Hodson et al., 1980) was greater than that seen after grafting pituitaries under the kidney capsule. This difference might be explained by the higher PRL levels found in the presence of the tumor. Recently, it has been suggested that the adrenals are involved in the modulatory role of PRL on the secretion of LH in pituitary-grafted male rats (Greeley & Kizer, 1979; Svare et al., 1979).
However, other investigators using pituitary grafts (McNeilly et al., 1980), the PRL- and GH-secreting tumor MtTW15 (Hodson et al., 1980), or the PRL- and ACTH-secreting tumor 7315a (Lamberts et al., 1981) found that the serum gonadotropin levels were as much reduced in the presence as in the absence of adrenals. The present study was carried out to investigate the possible effect of the adrenal on the regulation of gonadotropin secretion in the presence of the PRL- and ACTH-secreting tumor 7315a.

2. Materials and methods

2.1. Animals

Adult male rats of the Buffalo or the Buffalo x Wag/Rij strain were used. The animals were kept under conditions of controlled lighting (14 h of light and 10 h of darkness) and constant temperature (20-22°C). Pellet food (Hope Farms Standard Laboratory Diet) and tap water were provided ad libitum. Adrenalectomized animals received saline for drinking. Hyperprolactinemia was induced by the s.c. injection of 0.2 ml 7315a tumor tissue minced in saline (w/v, 1:2). Blood samples were collected by or-

bital sinus puncture under ether anesthesia. At the end of the experiments, the rats were killed by decapitation, and trunk blood was collected. Testes, seminal vesicles, prostates, and adrenals (if present) were then removed and weighed.

EXP. 1

On day 0, intact Buffalo x Wag/Rij rats were injected with a suspension of tumor 7315a. Untreated animals served as controls. On day 14, blood was collected. On day 34, the animals were decapitated, and trunk blood was collected.

EXP. 2

A group of Buffalo x Wag/Rij rats was gonadectomized and treated (s.c.) with a 1-cm long Silastic capsule (od, 0.1 cm) filled with testosterone. In Exp. 2, 3, and 4, the day of surgery was day 0. On day 4, half of the animals were injected with tumor suspension. Subsequently (days 10 and 24), blood was taken from the orbital plexus. On day 38, trunk blood was collected.

EXP. 3

After gonadectomy and adrenalectomy, a group of Buffalo rats received 1-cm long Silastic capsules filled with testosterone. On day 7, the tumor suspension was injected into half of the animals. Blood was taken by puncturing the orbital plexus on days 19 and 33 and by decapitation on day 47.

EXP. 4

Adrenalectomized Buffalo rats and sham-operated control animals were injected with tumor suspension on day 7. On days 13, 26, and 39, blood was taken.

2.2. Hormone assays

Serum levels of LH and FSH were determined using the antisera and procedures developed in our laboratory (Welschen et al., 1975). Serum levels of PRL were measured using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Concentrations of PRL, LH and FSH are expressed in terms of the standard NIAMDD reference preparations (NIAMDD-LH RP-1, NIAMDD-FSH RP-1, and NIAMDD-PRL RP-1). The interassay coefficients of variation were 9% for FSH, 11% for LH, and 15% for PRL. The amounts of hormone that reduced binding of labeled hormone to 90% of that occurring in the absence of unlabeled hormone were 6 ng FSH, 2 ng LH, and 0.4 ng PRL.

Serum levels of progesterone were determined by RIA using the method and antisera described previously (de Jong et al., 1974). Serum levels of testosterone were estimated using the method and antisera first described by Verjans et al. (1973). The interassay coefficients of variation were 13% for the progesterone assays and 15% for the testosterone assays. The percentage binding of radioactive steroid in the presence of 15 pg progesterone or testosterone in the corresponding assays was significantly ($p<0.01$) different from the control value.

2.3. Statistical analysis

The data were examined by analysis of variance, using the split plot design (Kirk, 1968); $P<0.05$ was adopted as the level of statistical significance.

TABLE 5.1 Body and organ weights of experimental and control rats.

	no. of rats	BW (g)	testis wt (mg)[a]	epididymis wt (mg)[a]	seminal vesicle (mg)[a]	prostate (mg)	adrenal wt (mg)[a]
Exp 1							
intact + tumor	7	274 ± 39[b]	2928 ± 51[b]	651 ± 25[b]	146 ± 13[b]	132 ± 13[b]	267 ± 58[b]
intact	7	336 ± 11	3527 ± 38	997 ± 15	339 ± 10	352 ± 16	40 ± 2
Exp 2							
gonadex + tumor	8	345 ± 10[b]		403 ± 18[c]	148 ± 4[b]	199 ± 14[b]	296 ± 13[b]
gonadex	6	408 ± 4		481 ± 12	335 ± 3	341 ± 21	50 ± 2
Exp 3							
adrenex + gonadex + tumor	7	348 ± 6		440 ± 12	260 ± 12	258 ± 18	
adrenex + gonadex	7	351 ± 7		457 ± 11	241 ± 15	240 ± 17	
Exp 4							
adrenex + tumor	6	328 ± 11[c]	2891 ± 39[b]	767 ± 15[b]	327 ± 28[b]	365 ± 22[b]	
intact + tumor	6	294 ± 5	2545 ± 42	589 ± 20	126 ± 8	145 ± 8	294 ± 7

Values shown are the mean ± S.E.M. Adrenex, adrenalectomized; gonadex, gonadectomized and treated s.c. with a Silastic capsule filled with testosterone. Tumor inoculations were carried out 32-40 days before autopsy.

[a] sum of the two organs

[b] $P<0.01$ vs control

[c] $P<0.05$ vs control

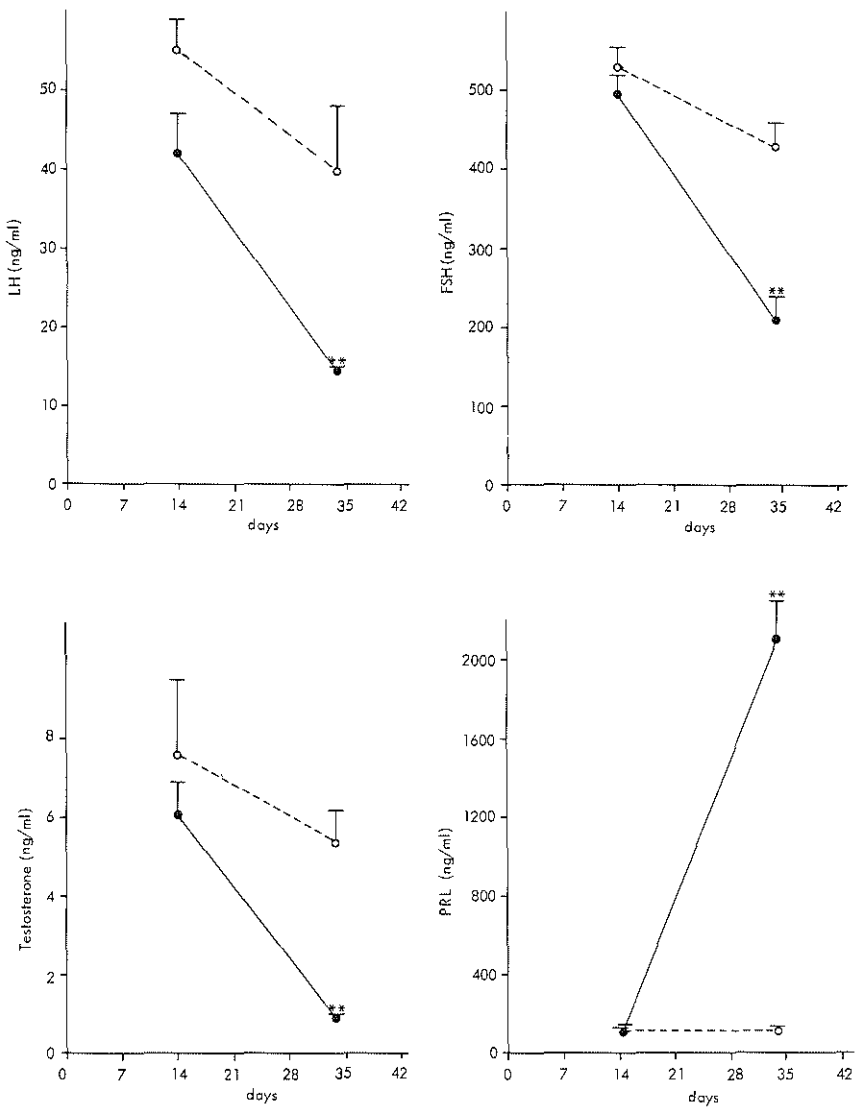

Fig. 5.1 Serum levels (mean ± SE) of LH, FSH, testosterone, and PRL in intact tumor-bearing rats (●–●) and intact control rats (o–o). Tumor inoculation was carried out on day 0. **, Significant differences between tumor-bearing rats and control rats ($P < 0.01$).

Significant interactions were tested with simple main effects. Means and S.E.M. values of body and organ weights were compared for significant differences by Student's t-test.

3. Results

EXP. 1: effects of tumor growth in intact male rats.

Serum levels (mean ± S.E.M) of PRL, LH, FSH, and testosterone are shown in Figure 5.1. By day 14 after tumor inoculation, PRL levels of tumor-bearing rats were as low as those in controls. Serum levels of LH, FSH, and testosterone determined in the same samples were not different in intact and tumor-bearing rats. However, the serum PRL concentrations 3 weeks later were significantly higher in tumor-bearing rats (2160 ± 203 ng/ml) than in controls (108 ± 19 ng/ml). Concentrations of LH, FSH, and testosterone were significantly lower in tumor-bearing animals than in controls. Mean serum concentrations of FSH, LH and testosterone in tumor-bearing rats were 2.0, 2.5, and 6.0 times lower than those found in control animals. Weights of testes, epididymides, seminal vesicles, and prostates were all significantly lower in the tumor-bearing animals than in controls (Table 5.1). In contrast, tumor-bearing rats had a significantly increased adrenal weight compared with that of the control animals.

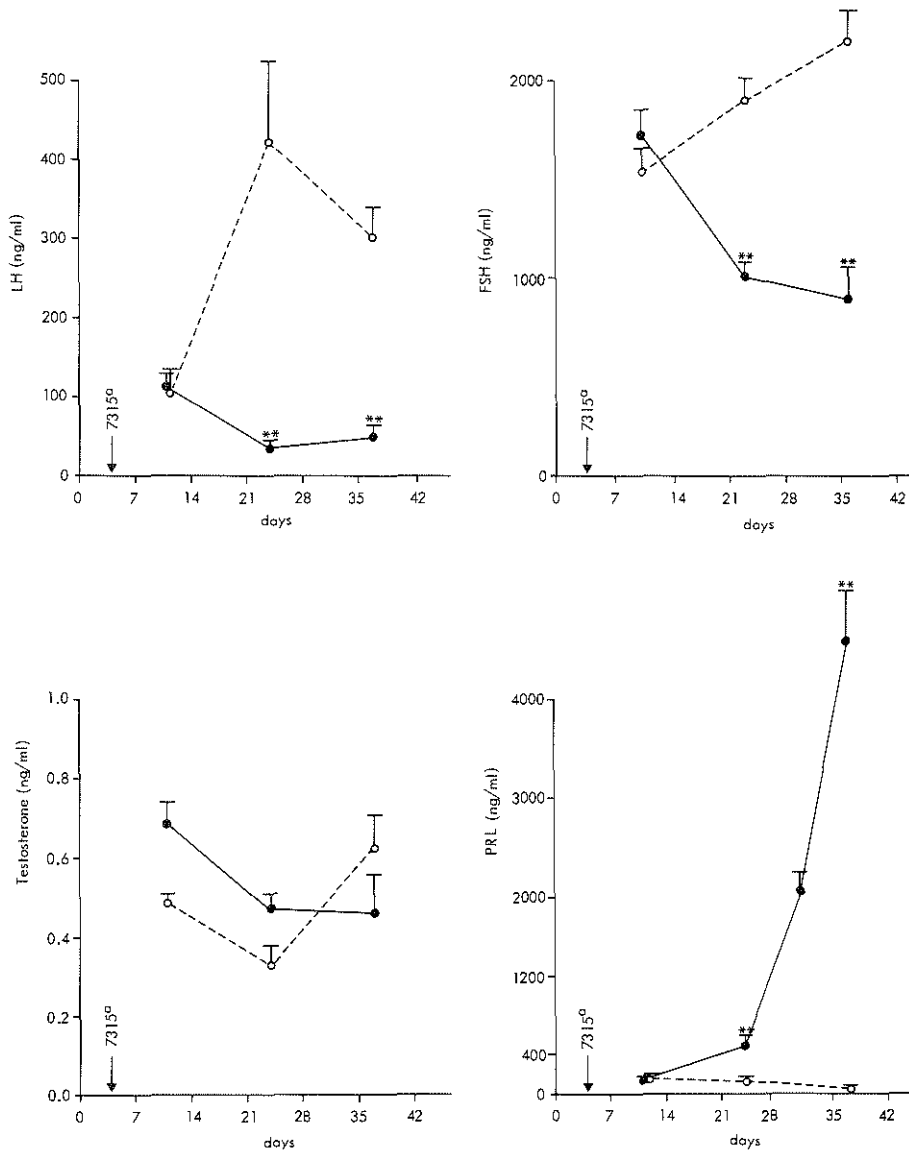

Fig. 5.2 Serum levels (mean ±SE) of LH, FSH, testosterone, and PRL in tumor-bearing, gonadectomized rats (●—●) and gonadectomized control rats (o--o). The gonadectomized rats were treated with small testosterone-filled capsules. The animals were gonadectomized on day 0. Tumor inoculation was carried out on day 4. **, Significant differences between tumor-bearing rats and control rats ($P < 0.01$).

EXP. 2: effects of tumor growth in gonadectomized, testosterone-treated male rats.

Six days after tumor inoculation, there were no significant differences in the serum levels of PRL, LH, FSH, and testosterone between control and tumor-bearing rats (Figure 5.2). However, on day 20 after tumor inoculation, the PRL level in tumor-bearing rats appeared to be significantly higher than that in control animals. Despite the absence of significant differences in testosterone concentrations in these serum samples, the concentrations of LH and FSH were significantly lower in tumor-bearing than in control rats. The observations made on day 34 after tumor inoculation were similar to those made on day 20 (Fig. 5.2). The weights of the epididymides, seminal vesicles, and prostates were significantly lower in tumor-bearing rats than in controls (Table 5.1), although the testosterone levels were not significantly different between groups. In a separate group of rats treated as described above, serum progresterone levels 4 weeks after tumor inoculation were significantly ($P<0.01$) higher in tumor-bearing rats (25.1 ± 4.1 ng/ml) than in control animals (0.6 ± 0.1 ng/ml).

EXP. 3: effects of tumor growth in adrenalectomized, gonadectomized, testosterone-treated male rats

During the course of the experiment, serum PRL levels increased considerably in tumor-bearing rats; no significant changes in PRL occurred in controls (Fig. 5.3).
Notwithstanding the large difference in serum PRL

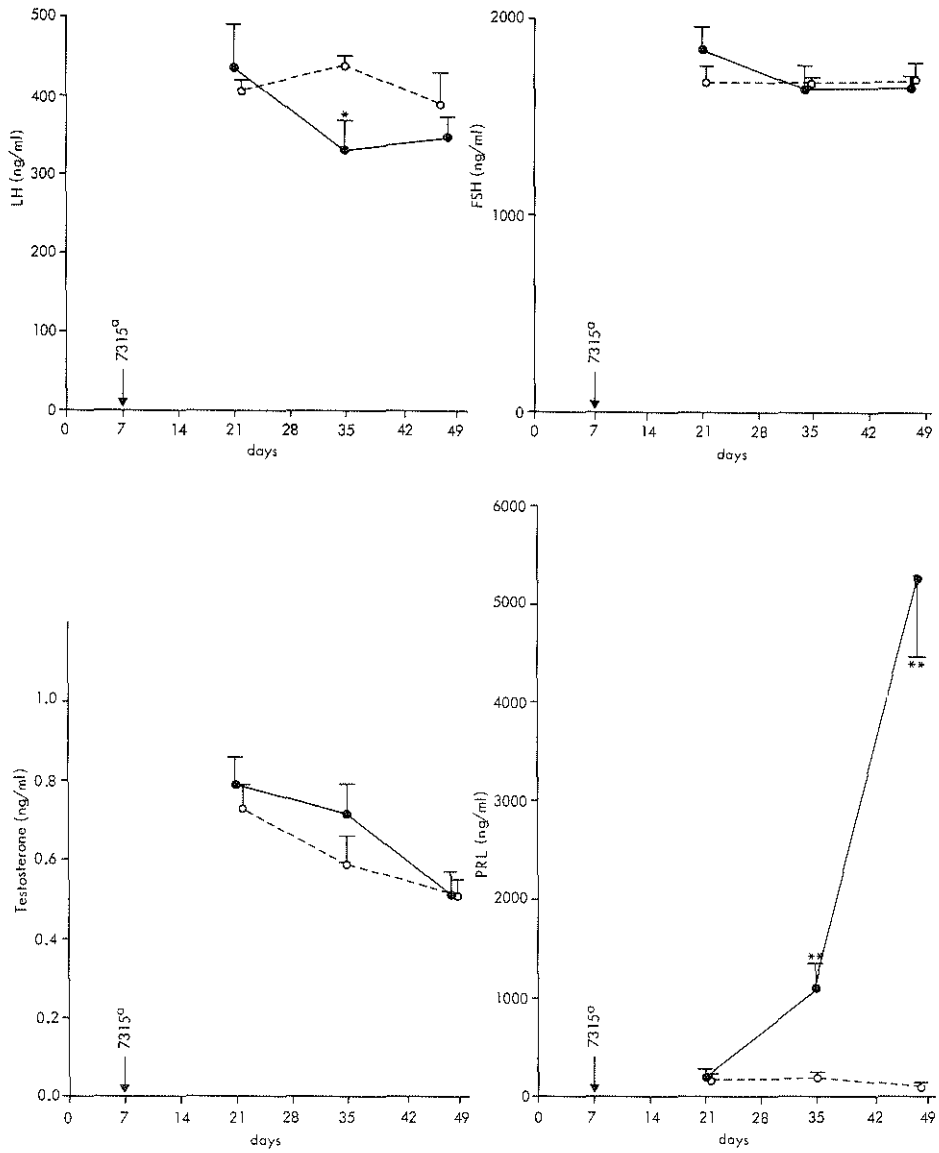

Fig. 5.3 Serum levels (mean ±SE) of LH, FSH, testosterone, and PRL in tumor-bearing, adrenalectomized and gonadectomized rats (●—●) and in adrenalectomized and gonadectomized control rats (o--o). The gonadectomized animals were treated with small testosterone-filled capsules. Surgery was carried out on day 0. Tumor inoculation was performed on day 7. Significant differences between tumor-bearing rats and control rats are indicated (**, $P < 0.01$; *, $P < 0.05$).

levels in tumor-bearing and control rats, the concentrations of LH and FSH were not significantly different between the two groups on day 42 after tumor inoculation. On day 27 after tumor inoculation, the tumor-bearing rats had a small but significant reduction in the serum LH level. On this day, however, the serum FSH concentration was as high in tumor-bearing rats as in controls. Testosterone concentrations were not significantly different between control and tumor-bearing rats. Serum progesterone concentrations were low in both tumor-bearing rats (0.6 ± 0.1 ng/ml) and control rats (0.9 ± 0.6 ng/ml). Furthermore, the weights of the epididymides, seminal vesicles, and prostates from tumor-bearing rats did not differ from those in controls (Table 5.1).

EXP. 4: effects of adrenalectomy in tumor-bearing male rats.

Six days after tumor inoculation, serum levels of PRL approximated those in control animals of previous experiments (Fig. 5.4). On days 19 and 32 after tumor inoculation, an increase in serum PRL was found in both intact and adrenalectomized rats. As in Exp. 1, serum levels of LH and FSH decreased significantly ($P<0.01$) in tumor-bearing intact rats during the course of the experiment. In contrast, in adrenalectomized tumor-bearing animals, LH and FSH concentrations remained unchanged. Although serum testosterone concentrations decreased significantly in both intact ($P<0.01$) and adrenalectomized ($P<0.05$) rats, the testosterone levels on days 19 and 32 were significantly lower in intact animals than in adrenalectomized rats. At autopsy, the weights of testes, epididymides, seminal vesicles, and prostates were

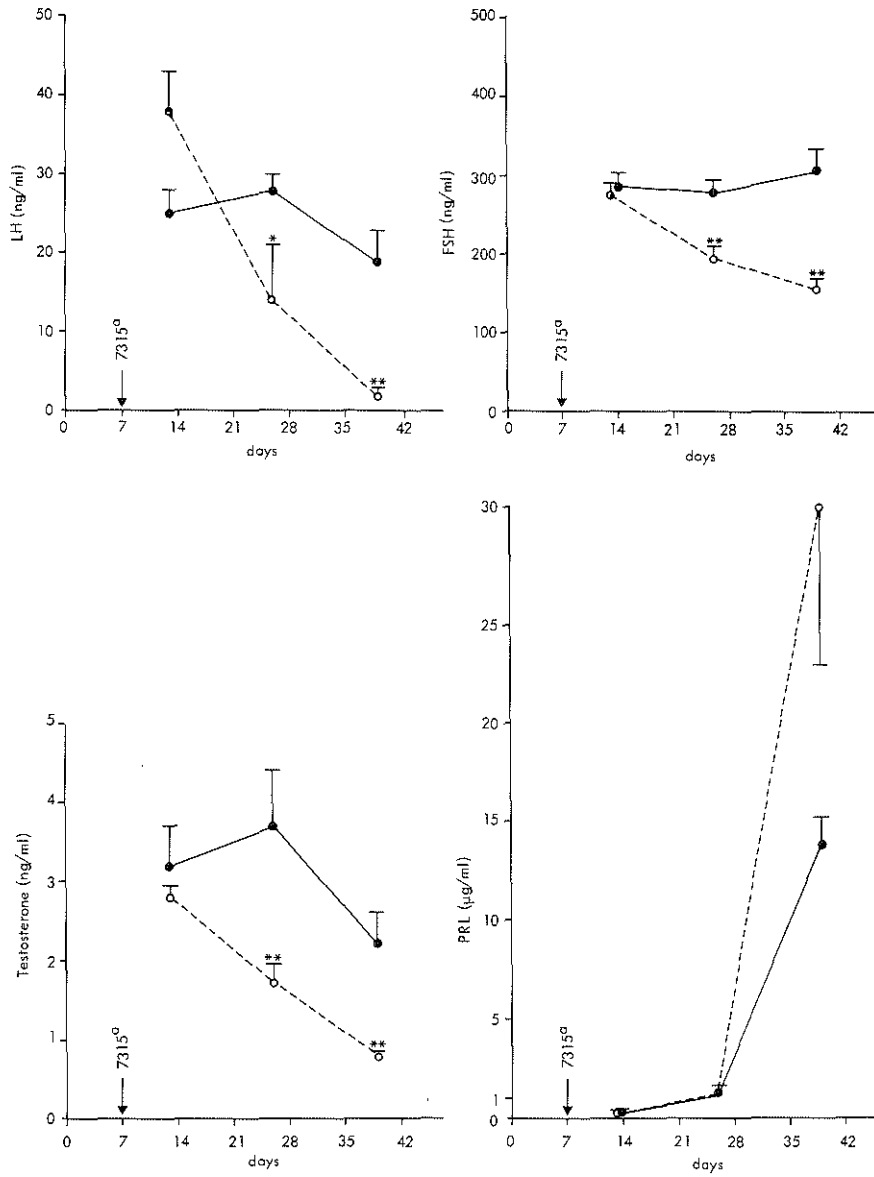

Fig. 5.4 Serum levels (mean ±SE) of LH, FSH, testosterone, and PRL in tumor-bearing, adrenalectomized rats (●—●) and tumor-bearing control rats (o--o). Adrenalectomy was performed on day 0. Tumor inoculation was carried out on day 7. Significant differences between tumor-bearing rats and control rats are indicated (**, $P < 0.01$; *, $P < 0.05$).

all significantly lower in intact tumor-bearing rats than in control rats (Table 5.1).

4. Discussion

In the present study, a major increase in the concentration of serum PRL in male rats was induced by the PRL- and ACTH-secreting tumor 7315a. At the end of the experiments, 5-6 weeks after tumor inoculation, PRL levels of several micrograms per ml were found. Similar high values of PRL in rats bearing tumor 7315a have also been found by other investigators (Cramer et al., 1979; Panerai et al., 1980; Lamberts et al., 1981). Since an association of hyperprolactinemia with low levels of LH and FSH is well established in intact male rats (Bartke et al., 1977; Celotti et al., 1978; McNeilly et al., 1978; Greeley & Kizer, 1979; Hodson et al., 1980), we were not surprised to find greatly reduced LH and FSH levels in intact tumor-bearing rats. However, in contrast to the results obtained in studies with animals grafted with normal pituitary tissue (Bartke et al., 1977; McNeilly et al., 1978), a decrease in both testosterone and weights of testes and accessory sex organs was found in the present and earlier investigations (Fang et al., 1974; Hodson et al., 1980) in which hyperprolactinemia was induced by PRL-producing tumors.

The combination of low serum LH and FSH levels with a low serum testosterone level was also found in our tumor-bearing, gonadectomized male rats. After gonadectomy, these animals received a testosterone-filled capsule which was too small to inhibit the postcastration rise in gonadotropins. Nevertheless,

when the tumor produced an increase in PRL, a further rise of serum LH and FSH was prevented. In fact, the elevated levels of serum gonadotropins were markedly reduced. Unexpectedly, the suppressive effects of the tumor on gonadotropin levels were completely absent in adrenalectomized animals. Even in the presence of serum PRL levels of about 5 μg/ml, the LH and FSH concentrations in gonadectomized, adrenalectomized, tumor-bearing rats were not different from those in gonadectomized, adrenalectomized rats. A similar observation was made in noncastrated adrenalectomized rats, in which a large increase in serum PRL induced by the tumor did not affect serum LH and FSH levels.

Our findings that high serum levels of PRL do not influence circulating LH and FSH in adrenalectomized rats are at variance with observations made by other investigators. Using the PRL- and GH-producing tumor MtTW15, Hodson et al. (1980) observed that the postcastration rise in serum LH was as much reduced in adrenalectomized, gonadectomized, tumor-bearing rats as in gonadectomized rats. Their data suggest that, regardless of the presence of the adrenals, high levels of PRL inhibit LH release. In addition, hyperprolactinemia induced by pituitary grafts (McNeilly et al., 1980) has been found to suppress gonadotropin levels in adrenalectomized rats. In the latter study however, the presence of the testes was required for pituitary grafts to exert an inhibitory effect on LH and FSH. The discrepancies between these findings (Hodson et al., 1980; McNeilly et al., 1980) and ours demonstrate that results obtained with transplants of normal pituitary tissue or tumor MtTW15 are different from those with tumor 7315a. Despite differences in the secretory activity of the various

types of PRL-producing transplants, it is difficult to explain why hyperprolactinemia induced in adrenalectomized male rats by pituitary grafts (McNeilly et al., 1980) or by pituitary tumor MtTW15 (Hodson et al., 1980) suppresses gonadotropin secretion while hyperprolactinemia elicited in similar animals by tumor 7315a does not.

Tumor 7315a secretes both PRL and ACTH (MacLeod et al., 1968). This resulted in adrenals which at autopsy were more than 6 times heavier than those in controls. It seems clear from the present data that secretory products of the tumor-stimulated adrenals are involved in the suppression of LH and FSH. This gonadotropin suppression might be due to ACTH, since the administration of ACTH to intact male rats can cause a reduction in testicular and accessory sex organ weights (Asling et al., 1951). Nevertheless, in the presence of the adrenals, PRL may also be involved in the suppression of LH and FSH. In gonadectomized, tumor-bearing rats, we measured high serum concentrations of progesterone, which were probably induced by stimulation of the adrenals by ACTH (Resko et al., 1969; Feder et al., 1971) or by ACTH plus PRL (Piva et al., 1973). Although progesterone alone does not affect gonadotropin levels in the rat (Caligaris et al., 1971; Nuti et al., 1977), there is evidence that this hormone together with PRL might have some inhibitory effect on LH and FSH. It has been found that the LH response to LRH is significantly depressed in hyperprolactinemic intact female rats (Vasquez et al, 1980). This phenomenon was attributed to the action of progesterone, since it was also found in hypeprolactinemic ovariectomized rats when progesterone was administered. Furthermore, Lamberts et al. (1981) reported that LH and FSH levels were

suppressed in adrenalectomized female rats bearing tumor 7315a. This observation is difficult to reconcile with our finding that FSH and LH were not inhibited in adrenalectomized males, unless we assume that high levels of PRL and progesterone secreted by the ovaries inhibited LH and FSH in females. An alternative possibility is that tumor 7315a suppresses serum gonadtropin levels in female rats without mediation of the secretory products of adrenals and ovaries.

In intact tumor-bearing rats, the weights of the prostate, seminal vesicles, and epididymides were markedly reduced compared to the values in controls. In part, this reduction must be the consequence of the decreased serum tetosterone concentration. However, the presence of the tumor also caused a reduction of sex accessory organ weights in gonadectomized, testosterone-treated animals in which testosterone levels were not different from those in the non-tumor-bearing gonadectomized rats. Since the inhibitory effects of the tumor on organ weights were completely absent in adrenalectomized animals, it seems likely that compounds secreted by the adrenals reduced the androgenic action of testosterone. One possible antiandrogenic substance is progesterone (Dorfman, 1967).

5. Summary

The presence of the transplantable PRL- and ACTH-secreting tumor 7315a in intact male rats resulted in very high serum PRL levels, decreased levels of LH and FSH, and reduced weights of testes and accessory sex organs. In gonadectomized rats bearing a small

testosterone-filled capsule, the tumor inhibited the postcastration rise in gonadotropins and reduced the weights of the prostate and seminal vesicles. However, after adrenalectomy of intact and gonadectomized male rats bearing a small testosterone filled capsule, inhibitory effects of the tumor on serum gonadotropin levels or on reproductive organ weights were totally absent. These results show that in adrenalectomized rats, hyperprolactinemia in itself does not affect gonadotropin secretion and the androgenic action of testosterone. Rather, the tumor might exert a gonadotropin inhibitory action through elevated levels of PRL combined with progesterone, which is secreted by the ACTH-stimulated adrenal.

6. References

Asling, C.W., Reinhardt, W.O. & C.H. Li. Effects of adrenocorticotropic hormone on body growth, visceral proportions, and white blood cell counts of normal and hypophysectomized male rats. Endocrinology 1951, 48, 534-547.

Bartke, A., Smith, M S , Michael, S.D , Peron, F.G. & S. Dalterio. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977, 100, 182-186.

Caligaris, L., Astrada, J.J. & S. Taleisnik. Biphasic effect of progesterone on the release of gonadotropin in rats. Endocrinology 1971, 89, 331-337.

Celotti, F., Massa, R. & L. Martini. Effect of prolactin on LH release in male rats. Neuroendocrinology 1978, 26, 41-49.

Cramer, O M , Parker, C.R. & J.C. Porter. Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinology 1979, 105, 636-640.

Dorfman, R.I. The antiestrogenic and antiandrogenic activities of progesterone in the defense of a normal fetus. Anat. Rec. 1967, 157, 547-557.

Fang, V.S., Refetoff, S. & R.L. Rosenfield. Hypogonadism induced by a transplantable, prolactin-producing tumor in male rats: hormonal and morphological studies. Endocrinology 1974, 95, 991-998.

Feder, H.H., Brown-Grant, K. & C.S. Corker. Pre-ovulatory progesterone, the adrenal cortex and the "critical period" for luteinizing hormone release in rats. J. Endocrinol. 1971, 50, 29-39.

Grandison, L., Hodson, C., Chen, H.T., Advis, J., Simpkins, J. & J. Meites Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology 1977, 23, 312-322.

Greeley, G.H. & J.S. Kizer. Evidence for adrenal involvement in the modulatory role of prolactin in luteinizing hormone secretion in the male rat. Endocrinology 1979, 104, 948-953

Gudelsky, G.A., Simpkins, J., Mueller, G.P., Meites, J. & K.E. Moore. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 1976, 22, 206-215

Hodson, C.A., Simpkins, J.W., Pass, K.A., Aylsworth, C.F., Steger, R.W. & J. Meites. Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinology 1980, 30, 7-10.

Jong, F.H. de, Baird, D T. & H J. van der Molen. Ovarian secretion rates of oestrogens, androgens and progesterone in normal women and in women with persistent ovarian follicles. Acta Endocrinol. 1974, 77, 575-587.

Kirk, R.E. Experimental design: procedures for the behavioral Sciences. Brooks/Cole, 1968. Belmont, California.

Lamberts, S.W.J., Zuiderwijk, J.M , Bons, E.G., Uitterlinden, P. & F.H. de Jong. Gonadotropin secretion in rats bearing a prolactin-secreting pituitary tumor: effects of naloxone administration. Fertil. Steril. 1981, 35, 557-562.

MacLeod, R.M., DeWitt, G.W. & M.C. Smith. Suppression of pituitary gland hormone content by pituitary tumor hormones. Endocrinology 1968, 82, 889-894.

McNeilly, A.S., Sharpe, R.M., Davidson, D.W. & H.M. Fraser. Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 1978, 79, 59-68.

McNeilly, A.S., Sharpe, R.M. & H.M. Fraser. Effect of adrenalectomy or castration on the inhibition of gonadtrophin secretion induced by hyperprolactinaemia in the adult male rat. J. Endocrinol. 1980, 85, 83-92.

Nuti, K.M. & H.J. Karavolas. Effect of progesterone and its 5α-reduced metabolites on gonadotropin levels in estrogen-primed ovariectomized rats. Endocrinology 1977, 100, 777-781.

Panerai, A.E., Sawynok, J., LaBella, F.S. & H.G. Friesen. Prolonged hyperprolactinemia influences β-endorphin and met-enkephalin in the brain. Endocrinology 1980, 106, 1804-1808.

Piva, F., Gagliano, P., Motta, M. & L. Martini. Adrenal progesterone: factors controlling its secretion. Endocrinology 1973, 93, 1178-1184.

Resko, J.A. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 1969, 164, 70-71

Svare, B., Bartke, A., Doherty, P., Mason, I., Michael, S.D. & M.S. Smith. Hyperprolactinemia suppresses copulatory behavior in male rats and mice. Biol. Reprod. 1979, 21, 529-535.

Vasquez, J.M., Ellegood, J.O., Nazian, S.J. & V.B. Mahesh. Effect of hyperprolactinemia on pituitary sensitivity to luteinizing hormone-releasing hormone following manipulation of sex steroids. Feril. Steril. 1980, 33, 543-549.

Verjans, H.L., Cooke, B.A., de Jong, F.H., de Jong, C.M.M. & H.J. van der Molen. Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid. Biochem. 1973, 4, 665-676.

Welschen, R., Osman, P., Dullaart, J., de Greef, W.J., Uilenbroek, J.Th.J. & F.H. de Jong. Levels of follicle stimulating hormone, luteinizing hormone, oestradiol-17β and progestesterone, and follicular growth in the pseudopregnant rat. J. Endocrinol. 1975, 64, 37-47.

Winters, S.J. & D.L. Loriaux. Suppression of plasma luteinizing hormone by prolactin in the male rat. Endocrinology 1978, 102, 864-868.

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CHAPTER 6

LRH LEVELS AND DOPAMINE LEVELS IN HYPOPHYSIAL STALK PLASMA AND THEIR RELATIONSHIP TO PLASMA GONADOTROPINS AND PRL LEVELS IN MALE RATS BEARING A PRL- AND ACTH-SECRETING PITUITARY TUMOR

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1. Introduction

During conditions of hyperprolactinemia plasma levels of LH and FSH are suppressed in female rats (Bartke et al., 1977; McNeilly et al., 1978; Lamberts et al., 1981). This phenomenon could be due to a direct effect of PRL on the gonadotropin-secreting cells since a blunted response of gonadotropins during stimulation with LRH has been observed in hyperprolactinemic rats (Winters et al., 1978; Vasquez et al., 1980). It is also possible that the inhibitory effect of PRL on the levels of gonadotropins could be mediated through the hypothalamus, perhaps resulting in a decreased release of LRH into the hypophysial portal vessels (Grandison et al., 1977; Hodson et al., 1981).

The secretion of PRL from the adenohypophysis in situ is inhibited after implantation of PRL into the hypothalamus or when plasma levels of PRL are elevated by ectopic pituitary grafts or PRL-secreting tumors (MacLeod, 1968; Mena et al., 1968; Voogt & Meites, 1971; Dang & Voogt, 1977; de Greef

& Zeilmaker, 1978; de Greef et al., 1980). This negative feedback action of PRL is probably exerted through altered hypothalamic release of neurohormones involved in the release of adenohypophysial PRL (Maltz et al., 1978; Voogt & Carr, 1974). Indeed, increased hypophysial stalk plasma levels of dopamine, a physiological PRL release-inhibiting factor secreted by the hypothalamus into the portal blood (MacLeod, 1976; Gibbs & Neill, 1978; Gibbs et al., 1979; de Greef & Neill, 1979; Neill et al., 1979), were observed in rats bearing a PRL-secreting tumor (Cramer et al, 1979; Gudelsky & Porter, 1980). It has been suggested that hypothalamic dopamine is involved in the regulation of hypothalamic LRH secretion (Hökfelt et al., 1972, McNeilly et al., 1978). Thus, the inhibitory effects of PRL on the secretion of gonadotropins may be caused by an effect on hypothalamic dopamine turnover (Gudelsky et al., 1976).

It was the aim of the present study to investigate the effects of hyperprolactinemia on the concentrations of LRH and dopamine in hypophysial stalk plasma of male rats. Hyperprolactinemia was induced by the transplantable 7315a tumor which secretes PRL and ACTH (MacLeod et al., 1968). Since it has been reported that in the male rat the adrenal glands are required for the effect of this tumor on the secretion of LH and FSH (Weber et al., 1982; Chapter 5), we also measured the levels of LRH and dopamine in hypophysial stalk plasma of tumor-bearing rats which were adrenalectomized at the time of tumor inoculation.

2. Materials and methods

2.1. Animals

Adult male rats of the Buffalo strain were used. They were kept under controlled conditions of temperature (20-22°C) and light (14 h light, 10 h dark schedule), and they had free access to pelleted food and tap water.

2.2. Experimental procedures

Hyperprolactinemia was induced by injecting the animals subcutaneously with 0.2 ml of a suspension of 7315a tumor tissue as reported before (Weber et al.; Chapter 5). Hypophysial stalk blood was collected 4-5 weeks after tumor inoculation. The animals were anesthetized with urethane (ethylcarbamate, Brocades ACF, Maarssen, The Netherlands; 1.1 - 1.2 μg/kg body weight, administered intraperitoneally as a 20% (w/v) solution in 0.9% NaCl and hypophysial stalk blood was collected at a rate of 6-10 l/min for 1 h as described by Porter & Smith (1967) with some modifications (Gibbs & Neill, 1978; de Greef & Neill, 1979; Neill et al., 1979). Before the hypophysial stalk was cut, 500 IU heparin (Thromboliquine, Organon, Oss, The Netherlands) was injected into a polyethylene cannula (0.58 mm internal diameter and 0.96 mm outside diameter) placed in the femoral artery and then a peripheral blood sample of about 1.5 ml was withdrawn from this arterial cannula for measurement of PRL, LH and FSH. In hypophysial stalk plasma dopamine and LRH were determined. Furthermore, the volume of hypophysial stalk plasma collected was meas-

ured allowing calculation of the secretion rates for dopamine and LRH per hour.

Two experiments were performed which were identical to those of the previous study (Chapter 5) so that direct comparisons between data from conscious (Chapter 5) and urethane-anesthetized (present study) rats could be made. In the first experiment, intact rats were inoculated with the tumor, while untreated intact rats served as controls. Each group contained 8 animals. In the second experiment, 20 rats were castrated and received a 0.5 cm long capsule of polydimethylsiloxane (Talas, Zwolle, The Netherlands: outside diameter 2.1 mm, inside diameter 1.5 mm) filled with testosterone (Steraloids Inc., Wilton N.H.), inducing plasma testosterone concentrations of about 1 ng/ml. 13 of these 20 animals were adrenalectomized, and tumor inoculation was carried out directly after the operation. Castration and adrenalectomy were performed while the rats were under ether anesthesia. Adrenalectomized rats received 0.9% NaCl solution (w/v) for drinking.

2.3. Hormone determinations

Concentrations of PRL, LH and FSH were measured by double-antibody radioimmunoassays in at least two dilutions of plasma as described earlier [PRL (de Greef & Zeilmaker, 1978); LH and FSH (Welschen et al., 1975)] using NIAMDD RP-1 as standards. Plasma levels of testosterone were estimated by radioimmunoassay (Verjans et al., 1973). Levels of dopamine were determined by a high-pressure liquid chromatographic-electrochemical method as described previously (Plotsky et al., 1978; de Greef et al., 1981).

Levels of LRH were measured by a double-antibody solid phase radioimmunoassay using an antiserum purchased from Miles-Yeda (Israel), produced and characterized according to Koch et al. (1973). The sensitivity of this assay is 3-5 pg and up to 200 microl of plasma were assayed for LRH. This assay did not detect immunoreactive LRH in peripheral plasma of male rats.

2.4. Statistical procedures

Results are presented as means $\pm$ S.E.M.. A nonparametric test (Mann-Whitney U test) was used to establish significant differences between the groups of animals. Differences were considered to be significant if $p<0.05$.

3. Results

3.1. Effect of 7315a tumor on levels of LRH and dopamine in hypophysial stalk plasma and on levels of LH, FSH and PRL in peripheral plasma in male rats

The results of this experiment are presented in Table 6.1.. In the tumor-bearing rats plasma levels of PRL increased 17-fold whereas the levels of LH and FSH in peripheral plasma were reduced by 45 and 70% respectively when compared to the controls. In the tumor-bearing animals the mean level of dopamine increased from 6 to 13 ng/ml, whereas the mean level of LRH decreased from 520 to 201 pg/ml in hypophysial stalk plasma.

TABLE 6.1 Plasma levels of PRL, LH and FSH and levels and secretion rates of dopamine and LRH in hypophysial stalk plasma of male rats anesthetized with urethane.[1]

treatment	PRL ng RP-1/ml	LH ng RP-1/ml	FSH ng RP-1/ml	dopamine ng/ml	ng/h	LRH pg/ml	pg/h
intact + tumor	1208 ± 319*	10.8 ± 0.3*	81.3 ± 4.5*	13.3 ± 1.8*	4.1 ± 0.6*	201 ± 16*	61.4 ± 4.9*
intact	72 ± 46	19.3 ± 3.1	251.4 ± 24.8	6.1 ± 0.8	1.4 ± 0.2	520 ± 48	122.4 ± 10.4

[1] 4-5 weeks earlier the experimental animals had been inoculated subcutaneously with 7315a tumor suspension. Control rats did not receive the tumor. Results are given as means ± S.E.M. Each group contained 8 animals.
* $P<0.05$

TABLE 6.2 Plasma levels of PRL, LH, and FSH and levels and secretion rates of dopamine and LRH in hypophysial stalk plasma of gonadectomized, testosterone-treated male rats anesthetized with urethane.[1]

treatment	animals n	PRL ng RP-1/ml	LH ng RP-1/ml	FSH ng RP-1/ml	dopamine ng/ml	ng/h	LRH pg/ml	pg/h
ADX	7	127 ± 32^a	162 ± 51^a	1047 ± 141^a	5.9 ± 0.9^a	1.8 ± 0.3^a	545 ± 51^a	165.0 ± 17.8^a
ADX + tumor	6	2827 ± 301^b	82 ± 14^a	951 ± 83^a	14.1 ± 2.0^b	4.5 ± 0.8^b	418 ± 57^a	132.8 ± 19.9^a
tumor	7	2393 ± 224^b	6 ± 2^b	212 ± 16^b	15.2 ± 1.2^b	4.3 ± 0.3^b	129 ± 31^b	36.1 ± 8.4^b

[1] 4 weeks before collection of blood the rats were inoculated subcutaneously with 7315a tumor and/or adrenalectomized (ADX). Results are given as means ± S.E.M.
^{a,b} means having different superscript are significantly different ($P<0.05$)

3.2. Effect of 7315a tumor on levels of LRH and dopamine in hypophysial stalk plasma and on levels of LH, FSH and PRL in peripheral plasma in adrenalectomized male rats

The results of this experiment are given in Table 6.2.. Levels of PRL in peripheral plasma were increased more than 20-fold in animals bearing the 7315a tumor. As in the first experiment, plasma levels of LH and FSH were suppressed in tumor-bearing rats which were not adrenalectomized. However, when the adrenal glands were removed before tumor inoculation, plasma levels of LH and FSH were not significantly different from those measured in adrenalectomized control animals. Levels of LRH in hypophysial stalk plasma did not differ significantly in adrenalectomized animals with or without the 7315a tumor, but were suppressed in tumor-bearing rats which were not adrenalectomized. Levels of dopamine in hypophysial stalk plasma increased in tumor-bearing animals with or without the adrenal glands when compared to animals without the tumor.

4. Discussion

Inoculation with the PRL- and ACTH-secreting 7315a tumor causes a suppression of peripheral levels of gonadotropins in both intact and castrated adult male rats. However, the tumor does not inhibit gonadotropin levels if adrenalectomy was carried out before tumor inoculation. Although in the present study the rats were anesthetized with urethane and underwent surgery in order to collect hypophysial stalk blood before a peripheral blood sample was taken, similar results as with conscious animals (Chapter 5) were

obtained. This finding that the anesthesia and surgical procedure did not interfere with the effect of the tumor on the gonadotropin levels allowed the study of the relationship between LRH and gonadotropins.

In this study the levels of LRH measured in hypophysial stalk plasma of urethane anesthetized male rats are about 5-10 times those reported previously by Fink & Jamieson (1967). The differences in secretion rates found in the two studies, however, are much lower (about 2-fold). The latter discrepancy might be due to the use of different antisera for measurement of LRH.

In the present study it was shown that suppression of gonadotropins in male rats by the 7315a tumor is probably caused by decreased levels of LRH in hypophysial stalk plasma, suggesting a suppressed release of LRH from the hypothalamus. Other authors have also suggested, on the basis of indirect evidence, that the release of LRH is decreased in hyperprolactinemic rats (Grandison et al., 1977; Gil-Ad et al., 1978; Hodson et al., 1980; Hodson et al., 1981). The conclusion that the decreased peripheral levels of LH and FSH are caused by a decreased release of LRH into the pituitary portal blood of the tumor-bearing animals is supported by the finding that a cause-and-effect relationship exists between hypothalamic LRH secretion and serum gonadotropin levels (Eskay et al., 1977). However, a decreased sensitivity of the pituitary gland to LRH cannot be ruled out as additional factor. The latter presumption is supported by the finding that stimulation of the release of LH and FSH during exogenous administration of LRH is blunted in hyperprolactinemic rats (Winter & Loriaux, 1978; Vasquez et al., 1980). It is possible that this decreased responsiveness of the

pituitary gland to LRH is caused by the long-term exposure to low levels of LRH. This might lead to a decreased content of LH and FSH in the pituitary gland or to a decreased number of pituitary LRH receptors. The first possibility does not seem likely since the levels of LH and FSH are increased in the pituitary glands of female rats bearing the same 7315a tumor (Lamberts et al., 1981). The second possibility seems more likely since Clayton et al. (1982) reported that in orchidectomized rats the number of pituitary LRH receptors are regulated in a positive manner by hypothalamic LRH. However, it is still controversial whether a decreased number of pituitary LRH receptors is of consequence for the responsiveness of the pituitary gland to LRH (Wagner et al., 1979; Ferland et al., 1981; Frager et al., 1981).

As in the previous study (Chapter 5) it was found that the effects of the tumor on the levels of LH and FSH were only present in animals with adrenal glands. Furthermore, the present study reveals that the presence of the adrenal glands is also necessary for the effect that the tumor exerts on the release of LRH. The mechanisms by which the adrenal gland affects the levels of LRH in hypophysial portal blood and the levels of LH and FSH in peripheral plasma have not yet been elucidated. Adrenal weights and the levels of progesterone and corticosterone in peripheral plasma are increased after tumor inoculation (unpublished results). It has been suggested that decreased sensitivity of the pituitary gland to LRH in hyperprolactinemic female rats might be a consequence of increased progesterone secretion in these animals (Vasquez et al., 1980). It is also possible that adrenal steroids other than progesterone are respon-

sible for the decreased release of LRH into the pituitary portal blood. This, however, requires further investigation.

Since the tumor 7315a secretes both PRL and ACTH, the suppression of LH and FSH might be due to PRL, ACTH or PRL plus ACTH. The observations that hyperprolactinemia induced by pituitary grafts has only a minor effect on pituitary-testicular function (Bartke et al., 1977; McNeilly et al., 1978) and that administration of ACTH to intact male rats causes a marked reduction in the weights of the testes and accessory sex organs (Asling et al., 1951) suggest that the effects of tumor 7315a are mainly due to ACTH.

It was observed in the present study that higher levels of dopamine in hypophysial stalk plasma were present in tumor-bearing animals than in control animals. Similar findings have been reported previously (Cramer et al., 1979; Gudelsky & Porter, 1980). In contrast to its effects on LRH levels in hypophysial portal blood, the tumor was also effective in altering dopamine levels in hypophysial stalk blood when the animals were adrenalectomized. It has been reported that PRL administration to ovariectomized rats leads to an increased dopamine turnover in the median eminence and anterior hypothalamus, suggesting that the activity of dopaminergic neurons is enhanced (Gudelsky et al., 1976), leading to elevated levels of dopamine in hypophysial stalk blood (Cramer et al., 1979; Gudelsky & Porter, 1980; present study). Hypothalamic dopamine has been suggested to be involved in the regulation of LRH secretion (Gudelsky et al., 1976; Hökfelt & Fuxe, 1972). Although morphological evidence is available that dopamine fibers might directly interact with LRH terminals (McNeill & Sladek, 1978), there is no substantial ev-

idence that changes in hypothalamic dopamine are important for altered secretion of gonadotropins. Also clinical studies are not conclusive in this respect. Judd et al. (1978) observed a reduction of plasma concentrations of LH in women receiving an infusion with dopamine. In contrast, treatment of hyperprolactinemic women with bromocriptine, a dopamine agonist, resulted in an increase of gonadotropins (Porter et al., 1981). The present data of adrenalectomized tumor-bearing animals are also not supportive for the view that the increased secretion of dopamine by dopamine neurons is an important factor for reduction in plasma levels of LH and FSH.

In conclusion, the present data clearly demonstrate that the suppression of peripheral gonadotropin levels in intact and castrated male rats bearing a PRL- and ACTH-secreting tumor can be explained by a decrease of LRH levels in hypophysial portal blood. However, the presence of the adrenal glands seems to be pivotal. Measurements of dopamine concentrations in hypophysial portal blood revealed an increase in tumor-bearing rats independent of the presence of the adrenal gland. It seems likely that the increased release of dopamine is caused by the high levels of PRL since PRL can affect its own secretion (see "Introduction") and long-term treatment of adrenalectomized rats with ACTH does not decrease the release of PRL (unpublished findings). Thus, PRL can influence the hypothalamic release of dopamine, an established PRL release-inhibiting factor (MacLeod, 1976; Gibbs & Neill, 1978; Gibbs et al., 1979; de Greef & Neill, 1979; Neill et al., 1979). It is furthermore suggested that the changes in dopamine cannot account for the inhibitory effect on the secretion of LH and FSH in hyperprolactinemic tumor-bearing rats.

5. Summary

The present study was concerned with the effects of a transplantable pituitary tumor secreting PRL and ACTH on the levels of LH and FSH in peripheral plasma and on the hypothalamic release of LRH and dopamine in the male rat. Male rats of the same age not inoculated with the tumor served as controls. Hypophysial stalk blood was collected from urethane-anesthetized rats 4-5 weeks after tumor inoculation to measure their LRH and dopamine content. A peripheral blood sample was withdrawn from the animals just before sectioning the hypophysial stalk to measure their content of LH, FSH and PRL. It was found that in the tumor-bearing rats the levels of PRL increased 17-fold, whereas plasma levels of LH and FSH decreased by 45 and 70% respectively, when compared with the control rats. In the tumor-bearing rats, the secretion rate of dopamine in hypophysial stalk plasma increased from 1.4 to 4.1 ng/h, whereas the secretion rate of LRH decreased from 122 to 61 pg/h. However, when at the time of tumor inoculation adrenalectomy was performed, the tumor did not decrease plasma levels of LH and FSH and the secretion of LRH into hypophysial stalk blood any longer. The effect of the tumor on hypothalamic dopamine secretion was, however, still present in the adrenalectomized rats.

It is concluded that the effect of the PRL- and ACTH-secreting pituitary tumor on plasma levels of LH and FSH requires the presence of the adrenal gland and that this effect is mediated through an inhibition of the hypothalamic release of LRH. Furthermore, this tumor increases the hypothalamic relese of dopamine independent of the presence of the adrenal gland.

6. References

Asling, C.W., Reinhardt, W.O. & C.H. Li. Effects of adrenocorticotrophic hormone on body growth, visceral proportions, and white blood cell counts of normal and hypophysectomized male rats. Endocrinology 1951, 48, 534-547.

Bartke, A., Smith, M.S., Michael, S.D., Peron, F.G. & S. Dalterio. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977, 100, 182-186.

Clayton, R.N., Channabasavaiah, K., Stewart, J.M. & K.J. Catt. Hypothalamic regulation of pituitary gonadotrophin-releasing hormone receptors: effects of hypothalamic lesions and a gonadotrophin-releasing hormone antagonist. Endocrinology 1982, 110, 108-115.

Cramer, O.M., Parker, C.R., Jr. & J.C. Porter. Secretion of dopamine into hypophysial portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinology 1979, 105, 636-640.

Dang, B.T. & J.L. Voogt. Termination of pseudopregnancy following hypothalamic implantation of prolactin. Endocrinology 1977, 100, 873-880.

Eskay, R.L., Mical, R.S. & J.C. Porter. Relationship between luteinizing hormone releasing hormone concentration in hypophysial portal blood and luteinizing hormone release in intact, castrated and electrochemically stimulated rats. Endocrinology 1977, 100, 263-270.

Ferland, L., Marchetti, B., Seguin, C., Lefebvre, F.A., Reeves, J.J. & F. Labrie. Dissociated changes of pituitary luteinizing hormone-releasing hormone (LHRH) receptors and responsiveness to the neurohormone induced by 17β-estradiol and LHRH in vivo in the rat. Endocrinology 1981, 109, 87-93.

Fink, G. & M.G. Jamieson. Immunoreactive hormone releasing factor in rat pituitary stalk blood: effect of electrical stimulation of the medial preoptic area. J. Endocrinol. 1976, 68, 71-87.

Frager, M.S., Pieper, D.R., Tonetta, S.A., Duncan, J.A. & J.C. Marshall. Pituitary gonadotropin-releasing hormone receptors. Effects of castration, steroid replacement, and the role of gonadotropin-releasing hormone in modulating receptors in the rat. J. Clin. Invest. 1981, 67, 615-623.

Gibbs, D.M. & J.D. Neill. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 1978, 102, 1895-1900.

Gibbs, D M., Plotsky, P.M., de Greef, W.J. & W.J. Neill. Effect of histamine and acetylcholine on hypophysial stalk plasma dopamine and peripheral levels. Life Sci. 1979, 24, 2063-2070.

Gil-Ad, I , Locatelli, V., Cocchi, D., Carminati, R., Arezzini, C. & E.E. Mueller. Effect of hyperprolactinemia and 2-Br-α-ergocryptine on neuroendocrine mechanism(s) for gonadotropin control. Life Sci. 1978, 23, 2245-2256.

Grandison, L., Hodson, C., Chen, H.T., Advis, J., Simpkins, J. & J. Meites. Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology 1977, 23, 312-322.

Greef, W.J. de & G.H. Zeilmaker. Regulation of prolactin secretion during the luteal phase in the rat. Endocrinology 1078, 102, 1190-1198.

Greef, W.J. de & J.D. Neill. Dopamine levels in hypophysial stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation. Endocrinology 1979, 105, 1093-1099.

Greef, W.J. de, van der Schoot, P. & G.H. Zeilmaker. Diurnal prolactin surges and sexual differentiation of the rat hypothalamus. Endocrinology 1980, 106, 486-489.

Greef, W.J. de & T.J. Visser. Evidence for the involvement of hypothalamic dopamine and thyrotrophin-releasing hormone in suckling-induced release of prolactin. J. Endocrinol. 1981, 91, 213-223.

Gudelsky, G.A. & J.C. Porter. Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. Endocrinology 1980, 106, 526-529.

Gudelsky, G.A., Simpkins, J., Mueller, G.P., Meites, J. & K.E. Moore. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 1976, 22, 206-215.

Hodson, C A., Simpkins, J.W., Pass, K.A., Aylsworth, C.F., Steger, R W. & J. Meites. Effects of a prolactin-secreting pituitary tumor on hypothalamic, gonadotropic and testicular function in male rats. Neuroendocrinology 1980, 30, 7-10.

Hodson, C.A., Burden, H.W., Louis, T.M., Poole, M. & I.E. Lawrence, Jr. Inhibition of hypothalamic LHRH depletion after ovariectomy by transplantable prolactin and growth-hormone-secreting tumor. Proc. Soc. Exp. Biol. Med. 1981, 167, 369-373.

Hökfelt, T. & K. Fuxe. Effects of prolactin and ergot alkaloids on the tubero-infundibular dopamine (DA) neurons. Neuroendocrinology 1972, 9, 100-122.

Judd, S.J., Rakoff, J.S. & S.S.C. Yen. Inhibition of gonadotropin and prolactin release by dopamine: effect of endogenous estradiol levels. J. Clin. Endocrinol. Metab. 1978, 47, 494-498.

Koch, Y., Wilchek, M., Fridkin, M., Chobsieng, P., Zor, U. & H.R. Lidner. Production and characterization of an antiserum to synthetic gonadotropin-releasing hormone. Biochem. Biophys. Res. Commun. 1973, 55, 616-622.

Lamberts, S.W.J., Zuiderwijk, J.M., Bons, E.G., Uitterlinden, P. & F.H. de Jong. Gonadotropin secretion in rats bearing a prolactin-secreting pituitary tumor: effects of naloxone administration. Fertil. Steril. 1981, 35, 557-562.

Login, I.S., Nagy, I. & R.M. MacLeod. Restoration of pituitary prolactin synthesis and release by the administration of morphine to rats bearing a transplanted prolactin-secreting tumor. Neuroendocrinology 1981, 33, 101-104.

MacLeod, R.M., De Witt, C.W. & M.C. Smith. Suppression of pituitary gland hormone content by pituitary hormones. Endocrinology 1968, 82, 889-894.

MacLeod, R.M.. Regulation of prolactin secretion. In: Frontiers in neuroendocrinology, volume 4. Eds. L. Martini & W.F. Ganong. Raven Press N.Y. 1976, 169-194.

Maltz, B.L., Buckman, M.T. & G.T. Peake. Studies on autoregulation of prolactin secretion from perifused rat pituitary glands in the basal and thyrotropin-releasing hormone-stimulated states. Endocrinology 1978, 103, 480-485.

McNeill, T.H. & J.R. Sladek, Jr. Fluorescenceimmunocytochemistry: simultaneous localization of catecholamines and gonadotropin-releasing hormone. Science 1978, 200, 72-74.

McNeilly, A.S , Sharpe, R.M., Davidson, D.W. & H M. Fraser. Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 1978, 79, 59-68.

Mena, F., Maiweg, H. & C.E. Grosvenor. Effect of ectopic pituitary glands upon prolactin concentration by the in situ pituitary of the lactating rat. Endocrinology 1968, 83, 1359-1362.

Neill, J.D., Plotsky, P.M. & W.J. de Greef. Catecholamines, the hypothalamus and neuroendocrinology applications of electrochemical methods. Trends Neurosci. 1979, 2, 60-63.

Plotsky, P.M., Gibbs, D.M. & J.D. Neill. Liquid chromatographic-electrochemical measurement of dopamine in hypophysial stalk blood of rats. Endocrinology 1978, 102, 1887-1894.

Porter, J.C. & K.R. Smith. Collection of hypophysial stalk blood in rats. Endocrinology 1967, 1182-1185.

Porter, J.C., Nansel, D.D., Gudelsky, G.A., Foreman, M.M., Pilotte, N.S., Parker, C.R., Jr., Burrows, G.H., Bates, G.W. & J.D. Madden. Neuroendocrine control of gonadotropin secretion. Fed. Proc. 1981, 39, 2896-2901.

Vasquez, J.M., Ellegood, J.O., Nazian, S.J. & V.B. Mahesh. Effect of hyperprolactinemia on pituitary sensitivity of luteinizing hormone-releasing hormone following manipulation of sex steroids. Fertil. Steril. 1980, 543-549.

Verjans, H.L., Cooke, B.A., de Jong, F.H., de Jong, C.M.M. & H.J. van der Molen. Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid. Biochem. 1973, 4, 665-676.

Voogt, J.L. & J. Meites. Effects of an implant of prolactin in the median eminence of pseudopregnant rats on serum and pituitary LH, FSH and prolactin. Endocrinology 1971, 88, 286-292.

Voogt, J.L. & L.A. Carr. Plasma prolactin levels and hypothalamic catecholamines synthesis during suckling. Neuroendocrinology 1974, 16, 108-118.

Wagner, T.O.F., Adams, T.E. & T.M. Nett. GNRH interaction with anterior pituitary. I. Determination of the affinity and number of receptors for GNRH in ovine anterior pituitary. Biol. Reprod. 1979, 20, 140-149.

Weber, R.F.A., Ooms, M.P. & J.T.M. Vreeburg. Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: a possible role of the adrenals. Endocrinology 1982, 111, 412-417.

Welschen, R., Osman, P., Dullaart, J., de Greef, W.J., Uilenbroek, J.Th.J. & F.H. de Jong. Levels of follicle stimulating hormone, luteinizing hormone, oestradiol-17 β and progesterone, and follicular growth in the pseudopregnant rat. J. Endocrinol. 1975, 64, 37-47.

Winters, S.J. & D.L. Loriaux. Suppression of plasma luteinizing hormone by prolactin in the male rat. Endocrinology 1978, 102, 864-868.

CHAPTER 7

APPENDIX TO CHAPTER 5 AND 6

EFFECTS OF ACTH ON SERUM GONADOTROPINS AND WEIGHTS OF TESTES AND ACCESSORY SEX ORGANS

1. Introduction

The experiments described in Chapters 5-6 have supplied evidence that the presence of the adrenals is obligatory for the 7315a tumor to exert its inhibitory action on gonadotropin secretion and on the weights of testes and accessory sex organs in male rats. This tumor secretes PRL, ACTH, β-endorphin and possibly other as yet unidentified products. It is difficult to assess from the previous work which of these hormones contributes to the stimulation of the adrenals in tumor-bearing animals.

In an attempt to differentiate between effects of PRL and ACTH, the effects of high doses of ACTH on serum gonadotropins and weights of testes and accessory sex organs of normal (non tumor-bearing) adult rats were studied.

2. Materials and methods

Adult male RxU rats were used, bred in our laboratory. Food, water and (after adrenalectomy) NaCl 0.9% (w/v) were available ad libitum. ACTH (Synacthen Depot) was given subcutaneously (10 U/day) dur-

ing 10 days to intact rats (Exp. 1), to gonadectomized rats with a subcutaneous implant of a 1 cm long testosterone-filled silastic capsule (Exp. 2), and to adrenalectomized animals (Exp. 3). Animals treated similarly but without ACTH served as controls. On the eleventh day, 18 hours after the last ACTH injection, the animals were sacrificed by decapitation. Trunk blood was collected and assayed for LH, FSH, and testosterone as described in Chapter 4 and for corticosterone by protein binding. The weights of testes, prostates, seminal vesicles and adrenals were noted.

Statistical analysis was by Student's t-test. $P<0.05$ was adopted to be significant.

3. Results

The results of the hormone determinations and organ weights are given in Table 7.1 and Table 7.2.
In intact animals treated with ACTH (Exp. 1) a significant suppression of LH, FSH and testosterone was observed, besides an increase of corticosterone levels. The weights of testes and accessory sex organs were significantly reduced. There was a 6-fold increase of adrenal weight.
In gonadectomized animals with a small testosterone filled silastic capsule (Exp. 2), which has been shown not to affect serum LH and FSH (Chapter 5), administration of ACTH resulted in a suppression of the post-castration rise of LH and FSH.
Despite similar levels of testosterone in the ACTH-treated gonadectomized and the gonadectomized control group, there was a significant reduction of

TABLE 7.1 Plasma levels of LH, FSH, testosterone and corticosterone of male rats treated with 10 U ACTH s.c. daily during 10 consecutive days.

	no. of rats	LH ng/ml	FSH ng/ml	testosterone ng/ml	corticosterone ng/ml
Exp. 1					
intact + ACTH	6	5.0 ± 0.0***	195 ± 10***	0.32 ± 0.04***	783 ± 29***
intact	5	14.4 ± 3.0	368 ± 35	4.52 ± 0.71	212 ± 26
Exp. 2					
gonadex + ACTH	8	5.0 ± 0.0***	346 ± 32***	1.39 ± 0.18	770 ± 52***
gonadex	8	151.5 ± 30.1	1098 ± 64	1.21 ± 0.07	188 ± 6
Exp. 3					
adrenex + ACTH	6	30.2 ± 2.2**	405 ± 31	3.67 ± 1.02	< 20
adrenex	6	41.8 ± 2.6	443 ± 19	5.47 ± 1.00	< 20

Values are given as means ± S.E.M. Gonadex, gonadectomized and treated s.c. with a silastic capsule filled with testosterone; adrenex, adrenalectomized.

** $P<0.01$ vs control

*** $P<0.001$ vs control

TABLE 7.2 Organ weights of male rats treated with 10 U ACTH s.c. daily during 10 consecutive days.

	no. of rats	testis° mg	prostate mg	seminal vesicle° mg	adrenal mg
Exp. 1					
intact + ACTH	6	2477 ± 86*	385 ± 30*	207 ± 10**	356 ± 24***
intact	5	2760 ± 97	483 ± 35	346 ± 6	63 ± 3
Exp. 2					
gonadex + ACTH	8		337 ± 9***	256 ± 7**	396 ± 24***
gonadex	8		437 ± 16	308 ± 15	60 ± 2
Exp. 3					
adrenex + ACTH	6	2651 ± 34	485 ± 11	328 ± 15	
adrenex	6	2750 ± 38	455 ± 16	317 ± 15	

Values are given as means ± S.E.M. Gonadex, gonadectomized and treated s.c. with a silastic capsule filled with testosterone; adrenex, adrenalectomized.

* $P<0.05$ vs control

** $P<0.01$ vs control

*** $P<0.001$ vs control

° Sum of the two organs

the weights of prostates and seminal vesicles of the ACTH-treated group.
Finally, no effects on FSH, testosterone and weights of testes and accessory sex organs were found in adrenalectomized animals treated with ACTH (Exp. 3). Although LH levels were significantly lower in the ACTH-treated than in the control group, this was not a consistent finding in repeated experiments: in a duplicate experiment the LH levels were 77.0 $\pm$ 11.3 and 34.5 $\pm$ 1.6 ng/ml (n=4) in adrenalectomized rats with (20 U during 10 consecutive days) and without ACTH treatment, respectively.

4. Discussion

Although the present experiments have not yet been completed, it can be concluded from the data presented that administration of high doses of ACTH to intact adult male rats leads to hypogonadotropic hypogonadism and decreased weights of testes and accessory sex organs. Furthermore, treatment with ACTH can suppress the post-castration rise of serum gonadotropins. The reduction of the weights of accessory sex organs in the presence of constant testosterone levels suggests a peripheral effect of products from the ACTH-stimulated adrenals on the handling of testosterone.
If adrenalectomy was carried out before the administration of ACTH, the suppressive effects on serum gonadotropin levels were no longer obtained. The weights of testes and accessory sex organs did not alter during treament with ACTH. From these observations it does not seem likely that ACTH has a direct effect on testes and accessory sex glands.

The present observations show that the presence of the adrenals is necessary for ACTH to cause a suppression of LH, FSH, testosterone and weights of testes and accessory sex organs. The similarity between these experiments and those with rats bearing the PRL- and ACTH-secreting tumor 7315a indicate that in tumor-bearing animals most of the effects may be due to ACTH.

It remains very tempting to examine which products from the tumor-stimulated or ACTH-stimulated adrenals are able, either alone or synergistically with pituitary hormones, to exert their suppressive action on gonadotropins, testes and accessory sex organs.

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CHAPTER 8

EFFECTS OF PRL ON TESTICULAR FUNCTIONS USING AN INTRATESTICULAR PITUITARY GRAFT

R.F.A. Weber, M.P. Ooms and J.T.M Vreeburg

1. Introduction

In male rats hyperprolactinemia induced by pituitary grafts results in suppression of gonadotropin secretion (Bartke et al., 1977; McNeilly et al., 1978). Despite these reduced plasma levels of LH, the plasma levels of testosterone have been found to be normal (Bartke et al., 1977, McNeilly et al., 1978). These data are in agreement with the observation that PRL enhances the sensitivity of the testis for LH (Bartke & Dalterio, 1976). Indeed, in hypophysectomized rats PRL maintains the capacity of the testis to bind LH (McNeilly et al., 1979; van Straalen & Zeilmaker, 1982) and potentiates the effect of LH on testicular testosterone secretion (Purvis et al., 1979). In contrast other investigators have found that Leydig cells from intact rats bearing pituitary transplants under their kidney capsules have an impaired capacity to secrete testosterone (Sharpe & McNeilly, 1979). This reduced steroidogenic capacity might be due to chronically suppressed serum gonadotropin levels as well as to a direct effect of PRL on Leydig cells (Sharpe & McNeilly, 1979).

In order to assess direct effects of PRL on the

testes, pituitaries were implanted in the left testes, and the secretion of testosterone and the content of testosterone and DHT of these pituitary grafted testes were compared with those in the right testes. In addition, tubuli seminiferi in the neighbourhood of the pituitary implant were inspected for histological changes.

2. Materials and methods

Adult male rats of the RxU strain, bred in our laboratory were caged under reversed lighting conditions (dark phase between 9.00 and 19.00 h) and in constant temperature (20-22^{o}C). Pelleted food (Hope Farms Standard Laboratory Diet) and tap water were always available. A pituitary from a female RxU rat was grafted under the tunica albuginea of the left testis, adjacent to the artery in the middle of the testis as indicated in Figure 8.1. In the right testis, a piece of cerebral cortex tissue was implanted. After 100 days 8 of these rats were sacrificed by decapitation Their testes were frozen and stored in liquid nitrogen. Further work-up of the testes consisted of slicing the frozen testes into 7 parts as shown in Figure 8.1. Directly after thawing the slices were homogenized in 0.9% NaCl (w/v) and stored at -20^{o}C for androgen determination. In the testes of 4 rats in addition to testosterone also PRL, LH and FSH were determined.
Another 5 rats were anesthetized with tribromoethanol (1.5 ml/100 gr BW 1:50 solution of tribromoethanol in saline, Avertine, Winthrop, i p.) 100 days after implantation of a pituitary in the left testis. From

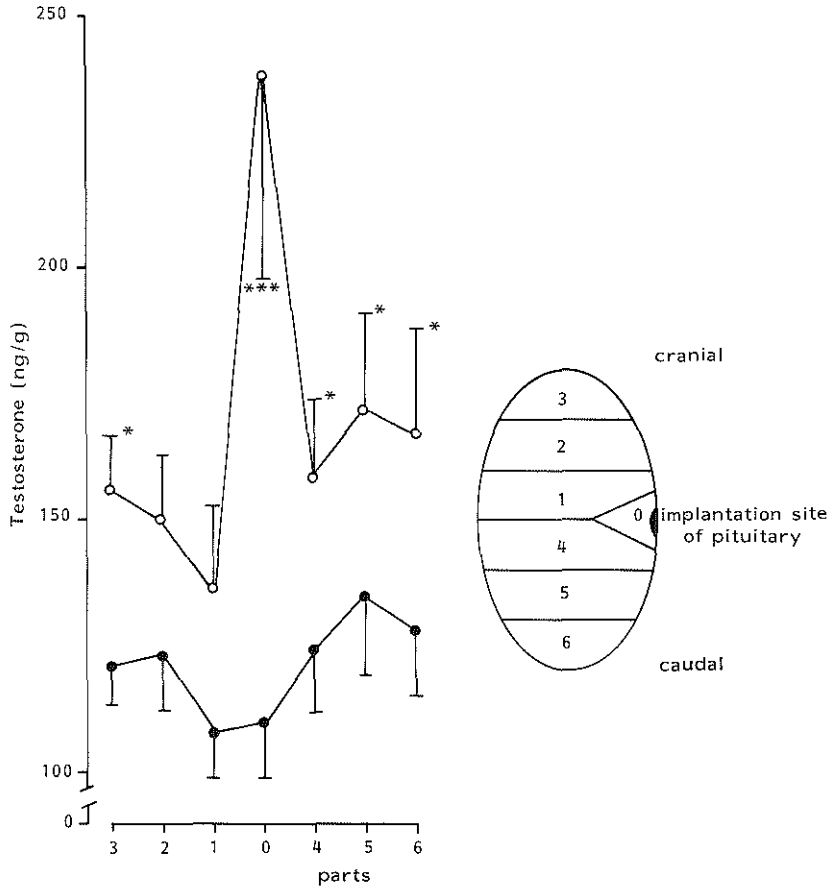

Fig. 8.1 Testosterone levels (ng/g. mean ± SEM) in 7 parts of the left (o—o) and right (●—●) testes 100 days after implantation of a pituitary in the left testes. The testes were divided as indicated.
*P<0.05 and ***P<0.001 compared with corresponding parts of the right testes.

these animals testicular venous blood was collected as described previously (de Jong et al., 1973). Directly after sampling testicular venous blood, the animals were killed by decapitation and trunk blood was collected The plasma samples were stored at -20°C for testosterone and PRL determinations. Subsequently the testes were removed and fixed in Bouin's fluid for histological examination.

2.1. Hormone assays

Serum levels of LH and FSH were determined using the antisera and procedures developed in our laboratory (Welschen et al., 1975). Serum levels of PRL were measured using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Concentrations of PRL, LH, and FSH are expressed in terms of the standard NIAMDD reference preparations (NIAMDD-LH RP-1, NIAMDD-FSH RP-1, and NIAMDD-PRL RP-1). The interassay coefficients of varation were 9% for FSH, 11% for LH, and 15% for PRL. The amounts of hormone that reduced binding of labeled hormone to 90% of that occurring in the absence of unlabeled hormone were 6 ng FSH, 2 ng LH, and 0.4 ng PRL.

Testosterone concentrations in plasma and testicular tissue were determined by RIA (Verjans et al., 1973). The inter-assay and intra-assay coefficients of variation were 15% and 7% respectively. In part 4 testosterone and DHT were measured after thin-layer chromatography. Separation of testosterone from DHT and from 5α-androstane-3α, 17β-diol was performed on aluminium oxide pre-coated thin layer plates in the system toluene-acetone 85:15. Concentrations of testosterone and DHT were determined by radioimmunoassay using methods as described above.

2.2. Statistical procedures

All data are given as mean $\pm$ S.E.M. Statistical analysis was done by Student's t-test or analysis of variance (two factorial, i.e. parts of testes and left or right testis, block design). Significant in-

teractions were tested with simple main effect. With significant F ratios, the differences between the means were tested with LSD (Kirk, 1968). Differences were accepted to be significant when $P<0.05$.

TABLE 8.1 PRL levels (ng/g) in parts of testicular tissue of left and right testes after implantation of 1 pituitary in the left testis (part 0).

parts°	left	right
3	290 ± 101	60 ± 39
2	188 ± 64	85 ± 38
1	593 ± 212	48 ± 23
0	22798 ± 18967***	318 ± 172
4	540 ± 208	80 ± 32
5	158 ± 71	90 ± 25
6	230 ± 93	90 ± 16

*** $P<0.001$

° parts as indicated in Figure 8.1

Values shown are means ± S.E.M.

3. Results

The PRL concentration in the left testes (321 ± 107 ng/g) without parts 0 was significantly ($P<0.05$; Student's t-test) higher than in the right testes (72 ± 25 ng/g). However, when corresponding parts of left and right testes were compared, it appeared that only the PRL levels in parts 0 were significantly different (Table 8.1).

Only in parts 0 the LH and FSH concentrations could be detected (9.4 ± 2.7 μg/g and 18.3 ± 7.4 μg/g respectively).

The testosterone levels measured in testicular tissue are shown in Figure 8.1. Comparison between corresponding parts of left and right testes showed

that testosterone levels were significantly higher in 5 of 7 parts of the left testes. In pieces of testicular tissue adjacent to the grafts (parts 4) testosterone and DHT were measured. It appeared that the mean levels of testosterone (119 ± 14 ng/g) and DHT (25 ± 6 ng/g) in parts 4 from the pituitary grafted testes were not significantly different from those (103 ± 14 ng testosterone/g; 17 ± 6 ng DHT/g) measured in the right testes.

TABLE 8.2 Levels of PRL (ng/ml) and testosterone (ng/ml) in testicular and peripheral venous plasma in rats, 100 days after implantation of a pituitary in the left testis.

	testicular venous plasma left	testicular venous plasma right	peripheral plasma	no. of rats
PRL	416 ± 118°*	59 ± 4.7*	41 ± 2.9	5
testosterone	84 ± 8.4	49 ± 1.5	1.04 ± 0.11	5

° $P<0.05$ vs right

* $P<0.05$ vs peripheral

Values shown are means ± S.E.M.

PRL and testosterone levels in peripheral and testicular venous plasma are given in Table 8.2. PRL levels in testicular venous plasma from the left testes were significantly higher than from the right testes ($P<0.05$) and from levels in peripheral venous plasma ($P<0.05$). Moreover PRL levels in testicular venous plasma from the right testes were significantly ($P<0.05$) higher than the levels in peripheral venous plasma. In contrast to the testicular testosterone concentrations no significant differences between the testosterone levels from the venous blood of left and right testes were found. As can be seen in Figure 8.2 the spermatogenesis in tubuli adjacent to the implanted pituitary did not show any alterations.

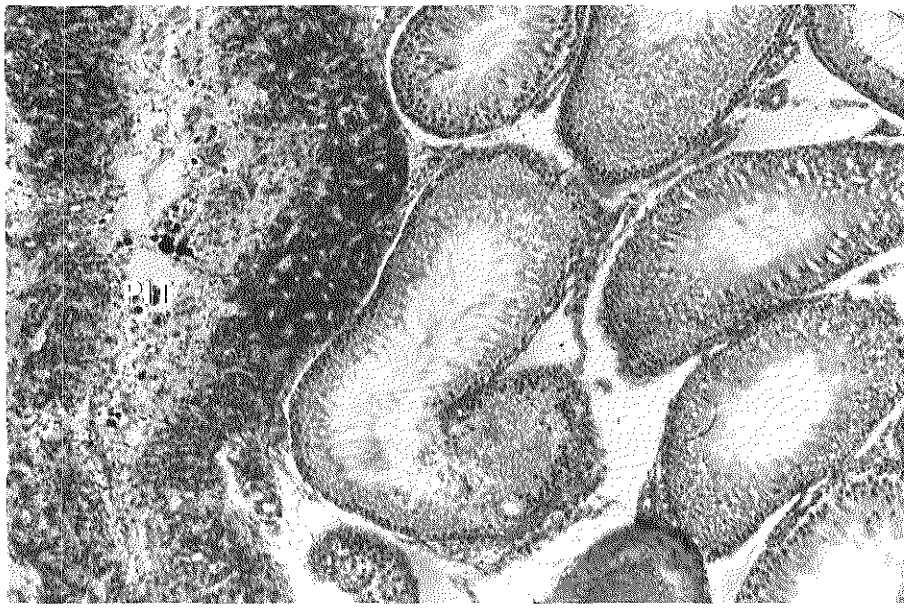

Fig. 8.2a Testicular tissue adjacent to pituitary tissue (PIT), 100 days after implantation of the pituitary into the testis (×50).

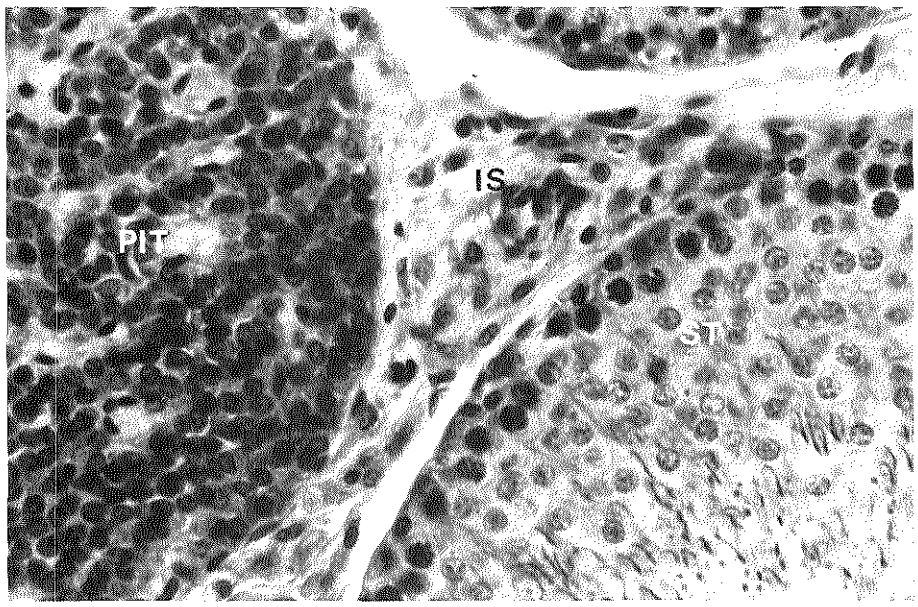

Fig. 8.2b Higher magnification (×320) of Fig. 8.2a, showing pituitary tissue (PIT), interstitial tissue (IS) and seminiferous tubuli (ST).

4. Discussion

Our data demonstrate that a pituitary implanted in the testis secretes appreciable amounts of PRL for a long period of time: 100 days after implantation of the pituitary into the testis, more than 400 ng PRL/ml was estimated in its venous plasma. The PRL levels in peripheral plasma, however, are not higher than those found normally in this strain of rats. This observation is in accordance with the finding that in the presence of only one pituitary under the kidney capsule, peripheral plasma levels of PRL are not significantly elevated (McNeilly et al., 1978). As could be expected, the wedge of testicular tissue which contained the pituitary graft, had a very high concentration of PRL and caused an increase in PRL in the left testis.

In addition to the rise in the testicular concentration of PRL, als testosterone increased. Since the rise in the tissue testosterone concentration was not accompanied by a rise in testosterone secretion, an increase in the binding capacity for testosterone might have occurred. The increase in testicular testosterone concentration might have been due to PRL although LH secretion by the pituitary graft cannot be ruled out completely, since the pituitary grafted part of the testis contained appreciable amounts of LH. In the other parts of the left testis, the LH concentrations were below the limit of detection. This observation and the fact that testosterone in concentrations much lower than present in the testis are capable to inhibit the hypophysial LH secretion (Drouin & Labrie, 1976) makes it probable that the LH secretion by the pituitary grafts have been negligible.

In a variety of studies, PRL has been shown to inhibit the 5α-reductase activity in the adrenal (Witorsch & Kitay, 1972), but the effects of PRL on 5α-reductase activity in accessory sex glands are inconclusive (Yamanaka et al., 1975; Manandhar & Thomas, 1976; Prins & Lee, 1982). The present study demontrates clearly that in testicular tissue adjacent to the pituitary graft the ratio between testosterone and DHT is not changed, suggesting a normal 5α-steroid reductase activity despite elevated PRL levels.

The observations that spermatogenesis in tubuli adjacent to the implanted pituitary seemed to be normal, and that the steroidogenic capacity of the testis was not inhibited, are in accordance with our previous study (Chapter 5) in which was reported that extremely high PRL levels due to a PRL- and ACTH-secreting tumour do not affect testicular weight or serum testosterone in adrenalectomized rats.

5. Summary

Pituitary glands were grafted under the capsule of the left testis to induce high levels of PRL in this organ. 100 Days after implantation, significantly increased levels of PRL were found in the tissue and the venous plasma of the left testis. Although the levels of testosterone in testicular venous plasma were not elevated, the testicular content of testosterone was increased when compared to the right testis.

The ratio of testosterone and DHT was not affected in the pituitary grafted testis.

Since the tubuli seminiferi adjacent to the pitui-

tary graft appeared to be completely normal, it is concluded that in the rat high levels of PRL have no direct inhibitory effect on testicular functions.

6. References

Bartke, A. & S. Dalterio. Effects of prolactin on the sensitivity of the testes to LH. Biol. Reprod. 1976, 15, 90-93.

Bartke, A., Smith, M.S., Michael, S.D., Peron, F.G. & S. Dalterio. Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977, 100, 182-186.

Drouin, J. & F. Labrie. Selective effect of androgens on LH and FSH release in anterior pituitary cells in culture. Endocrinology 1976, 98, 1528-1534.

Jong, F.H. de, Hey, A.H. & H.J. van der Molen. Effect of gonadotrophins on the secretion of oestradiol-17β and testosterone by the rat testis. J. Endocrinol. 1973, 57, 277-284.

Kirk, R.E. Experimental design: procedures for the behavioral sciences. Brooks/Cole, 1968. Belmont, California.

McNeilly, A.S., Sharpe, R.M., Davidson, D.W. & H.M. Fraser. Inhibition of gonadotrophin secretion by induced hyperprolactinaemia in the male rat. J. Endocrinol. 1978, 79, 59-68.

McNeilly, A.S., de Kretser, D.M. & R.M. Sharpe. Modulation of prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion by LHRH and bromocriptine (CB154) in the hypophysectomized pituitary-grafted male rat and its effect on testicular LH receptors and testosterone output. Biol. Reprod. 1979, 21, 141-147.

Manandhar, M.S.P. & J.A. Thomas. Effect of prolactin on the metabolism of androgens by the rat ventral prostate gland in vitro. Invest. Urol. 1976, 14, 20-22.

Prins, G.S. & C. Lee. Influence of prolactin-producing pituitary grafts on the in vivo uptake, distribution, and disappearance of [^{3}H]testosterone and [^{3}H]dihydrotestosterone by the rat prostate lobes. Endocrinology 1982, 110, 920-925.

Purvis, K., Clausen, O.P.F., Olsen, A., Haug, E & V. Hansson. Prolactin and Leydig cell responsiveness to LH/hCG in the rat. Arch Androl. 1979, 3, 219-230.

Sharpe, R.M. & A.S. McNeilly. The effect of induced hyperprolactinaemia on Leydig cell function and LH-induced loss of LH-receptors in the rat testis. Mol. Cell. Endocrinol. 1979, 16, 19-27.

van Straalen, R.J.C. & G H. Zeilmaker. Observations on the effects of prolactin on LH-receptors and steroidogenesis in corpus luteum and testis of the hypophysectomized rat. Acta Endocrinol. 1982, 99, 437-442.

Verjans, H.L., Cooke, B.A., de Jong, F.H., de Jong, C.M.M. & H.J. van der Molen. Evaluation of a radioimmunoassay for testosterone estimation. J. Steroid. Biochem. 1973, 4, 665-676.

Weber, R.F.A., Ooms, M.P. & J.T.M. Vreeburg. Effects of a prolactin- and adrenocorticotropin-secreting tumor on gonadotropin levels and accessory sex organ weights in adult male rats: a possible role of the adrenals. Endocrinology 1982, 111, 412-417.

Welschen, R., Osman, P., Dullaart, J., de Greef, W.J., Uilenbroek, J.Th.J. & F.H. de Jong. Levels of follicle stimulating hormone, luteinizing hormone, oestradiol-17β and progesterone, and follicular growth in the pseudopregnant rat. J. Endocrinol. 1975, 64, 37-47.

Witorsch, R.J. & J.I. Kitay. Pituitary hormones affecting adrenal 5α-reductase activity: ACTH, Growth Hormone and prolactin. Endocrinology 1972, 91, 764-769.

Yamanaka, H., Kirdani, R.Y., Saroff, J., Murphy, G.P. & A A. Sandberg. Effects of testosterone and prolactin on rat prostatic weight, 5α-reductase, and arginase. Am. J. Physiol. 1975, 229, 1102-1109.

SUMMARY

In this thesis some effects of PRL on reproductive functions have been investigated in men with a PRL-secreting pituitary adenoma. For comparison an animal model has been used:

- In rats hyperprolactinemia has been induced by subcutaneous inoculation of a PRL- and ACTH-secreting transplantable pituitary tumor.
- The local effects of PRL on testicular functions have been studied by implantation of a pituitary in a testis.

Hyperprolactinemia in man

The symptoms of hyperprolactinemia in men with a pituitary adenoma are, as indicated in many reports (Chapter 1), mostly due to local effects of the tumor on para- and suprasellar tissues. Patients may present with headache, impaired vision, visual field defects, paresis of the eyemuscles and hypopituitarism. Loss of libido and potency, which symptoms may retrospectively be present for a long period, are considered by many authors to be typical features for male hyperprolactinemia. Gynecomastia and galactorrhea are uncommon findings. Much of the research with respect to function of the hypothalamic-pituitary-testicular axis in hyperprolactinemic men reported over the past few years is still controversial. The basal serum levels of LH and FSH may be normal or decreased and their response to LRH may be normal or blunted. Serum testosteron levels have

been found subnormal, even in the presence of normal basal and LRH-stimulated LH and FSH levels. Furthermore serum testosterone levels may show a normal response to the administration of hCG. It is difficult to draw conclusions from the data presented in the various papers since most of the reported series comprised men with macroadenomas or men who had been treated before the time of presentation.

In Chapter 3 the findings of 32 men with a PRL-secreting pituitary adenoma have been discussed. A macroadenoma was present in 19 patients, who presented with headache, visual field defects and/or impaired vision and hypopituitarism. The majority of these 19 men had loss of libido or impotence. Gynecomastia and galactorrhea were present in three of them. In the presence of high serum PRL levels, the basal levels of LH and FSH were normal but their response to LRH was blunted. Furthermore low serum testosterone levels were found in most of the patients. It was not possible to show whether the suppression of the pituitary-gonadal axis in these 19 patients with a macroadenoma was due to the hyperprolactinemia or to the loss of function of the gonadotropic cells by compression and/or destruction caused by the tumor itself. In support of this last presumption is the loss of other anterior pituitary functions in these 19 men harboring a macroadenoma. Loss of pituitary-thyroidal function, measured by basal and TRH-stimulated TSH levels, subnormal levels of 11-deoxycortisone after the administration of metyrapone representing an insufficient pituitary-adrenal axis and panhypopituitarism have been observed. The other 13 out of 32 hyperprolactinemic men had been selected from a group of 598 infertile

men with sperm abnormalities. The low incidence of hyperprolactinemia in our series of infertile men was a confirmation of the literature. Only 2 of these 13 patients suffered from diminished libido. The others had no complaints of impotence or loss of libido. The 2 patients with diminished libido had a macroadenoma, the others a microadenoma. PRL levels in this group of 13 patients were much lower compared to the first group of 19 men. Gynecomastia and galacthorrhea were present in 2 patients. In contrast to the findings in the first group, the anterior pituitary functions and especially the basal and LRH-stimulated LH and FSH levels were normal. Furthermore testosteron levels were normal in most of the patients. In this group of infertile patients with microadenomas, that probably cannot compress and/or destruct normal pituitary tissue, the only slightly increased PRL levels are not able to suppress the pituitary-gonadal axis. Nevertheless treatment with bromocriptine was given to study the effects of normalising PRL levels on fertility. Two patients fertilized before, 4 men during treatment. None of these 4 men showed improvement of their sperm qualities, while testosterone levels significantly increased as has been reported in the literature. Another common finding was the restoration of libido in the 2 men with a macroadenoma in the group of infertile patients.

We have suggested that according to our findings the suppression of the pituitary-gonadal axis in patients with a PRL-secreting macroadenoma is mainly due to local compression and/or destruction of normal pituitary tissue. Improvement of libido and increment of testosterone levels during PRL-reducing therapy may indicate an effect of PRL.

Subsequent to possible effects of hyperprolactinemia on libido in man, copulatory behavior has been studied in hyperprolactinemic male rats (Chapter 4). Hyperprolactinemia has been induced by the inoculation of the PRL- and ACTH-secreting tumor 7315a subcutaneously. Two Weeks after inoculation serum PRL levels begin to rise up to several micrograms per ml 5 to 6 weeks after inocultion.

The presence of the tumor caused hypogonadotropic hypogonadism in adult male rats (Chapter 5). The decreasing testosterone levels during growth of the tumor forced us to study copulatory behavior in castrated animals with constant testosterone levels by placing subcutaneously a testosterone filled silastic tube. Increment of PRL concentrations induced a remarkable change of copulatory behavior. The number of mounts increased very much and the time which elapsed between the first mount and ejaculation increased. The number of intromissions before ejaculation did not change. The increased number of mounts was probably due to a relative inability to intromit properly during hyperprolactinemia.

Since the tumor secretes both PRL and ACTH, resulting in large adrenals (Chapter 5) we have also studied copulatory behavior of adrenalectomized tumor-bearing animals. Copulatory behavior of adrenalectomized tumor-bearing animals, with very high serum PRL levels, and of adrenalectomized control animals did not differ.

From these observations can be concluded that even very high PRL levels have no effect on copulatory behavior of the adrenalectomized male rat.

With respect to the effects of PRL on serum LH,

FSH and testosterone levels in the male rat two different observations have been described in the literature (Chapter 2). A moderate increase of serum PRL level, induced by ectopic pituitaries, causes suppression of LH- and FSH-concentrations, while serum testosterone levels remain normal. Moreover, ectopic pituitaries are able to suppress the post-castration rise of LH and FSH. The inoculation of PRL-secreting tumors is accompanied with very high PRL levels, atrophy of the testes and accessory sex glands and suppression of LH, FSH and testosterone.

In Chapter 5 levels of LH, FSH and testosterone and the weights of testes, accessory sex glands and adrenals in adult male rats with the PRL- and ACTH-secreting tumor 7315a are reported and have been discussed.

Tumor-inoculation in intact animals resulted in a decrease of serum LH, FSH and testosterone levels and in a decrease of the weights of testes and accessory sex glands. The weights of the adrenals were very much increased. Furthermore mammary gland tissue was fully developed and contained a lot of milk. The tumor was also able to suppress the post-castration rise of LH and FSH. If adrenalectomy was carried out before tumorinoculation the rise of LH and FSH after castration was normal, while LH, FSH and testosterone levels did not change in adrenalectomized tumor-bearing animals despite very high serum PRL levels. The observations in castrated animals have been done in the presence of constant subnormal testosterone concentrations, caused by subcutaneously placed testosterone-filled silastic capsules. Although comparable testosterone levels were present in adrenalectomized and non-adrenalectomized tumor-bearing rats, at autopsy the weights of accessory sex

glands were decreased in the latter group.

The regulation of LH- and FSH-secretion in tumor-bearing animals have been further investigated (Chapter 6) by determinations of dopamine and LRH in hypophysial stalk blood. Numerous studies have shown that the secretion of hypothalamic dopamine, the most important "PRL-inhibiting factor", is regulated by PRL via a short-loop positive feed-back mechanism. Moreover, some authors state that dopamine is involved in the secretion of LRH by the hypothalamus. Disturbances in the secretion of LH and FSH during hyperprolactinemia might be explained by a suppressive action of dopamine The decrease of LH and FSH in intact rats bearing the 7315a tumor might be explained by a decrease of LRH in hypophysial stalk blood. Both the dopamine concentration and the secretion rate of dopamine in hypophysial stalk blood were increased. Similar serum concentration of LH and FSH were found in tumor-bearing and non-tumor-bearing rats if adrenalectomy was performed before tumor-inoculation. Also the LRH concentrations in hypophysial stalk blood were not different. Dopamine levels, however, were increased in hypophysial portal blood of tumor-bearing animals.

The results of the experiments described in Chapter 5 and 6 indicate that hyperprolactinemia in adrenalectomized animals bearing the PRL- and ACTH-secreting 7315a tumor is not accompanied by a suppression of LH and FSH. In Chapter 7 is described the investigation as to which factor from the 7315a tumor, which secretes besides PRL and ACTH at least β-endorphin, is able to suppress the secretion of LH and FSH in the presence of the adrenals. The administration of ACTH subcutaneously to intact adult male rats caused a suppression of serum LH, FSH and

testosterone and a decrease of the weights of testes and accessory sex glands. Moreover, the administration of ACTH was able to suppress the post-castration rise of LH and FSH. In castrated rats with comparable testosterone levels obtained by the previously mentioned testosterone implantation, the weights of accessory sex gland of the ACTH-treated animals were less than those of the non-treated rats. These findings are similar to those in animals bearing the 7315a tumor as described in Chapter 5 and 6.

Although hyperprolactinemia in adrenalectomized animals with the 7315a tumor did not influence serum testosterone concentrations (Chapter 5), in the literature (Chapter 2) stimulatory effects of PRL on gonadal function of the rat have also been described.

In Chapter 8 gonadal functions have been further investigated. Implantation of a pituitary in 1 testis caused high PRL levels both in testicular tissue and in testicular venous blood from the testis with the pituitary transplant. Hyperprolactinemia was not present in peripheral blood. 100 Days after implantation the testosterone levels in testicular tissue of the whole testis with the pituitary implant were higher than in the other testis. Testosteron concentrations did not change either in testicular venous blood or in peripheral venous blood. Spermatogenesis even in tubuli adjacent to the pituitary graft seemed to be completely normal.

The experiments presented in this thesis in general do not support the accepted opinion that PRL itself either in man or in the male rat is able to disturb reproduction. In the male rat bearing the PRL- and ACTH-secreting tumor 7315a the presence of the adrenals is obligatory to suppress serum LH, FSH and testosterone concentrations. Furthermore, adrenals

stimulated with pharmacological doses of ACTH are also able to suppress LH, FSH and testosterone secretion. Moreover, the stimulatory effects of testosterone on accessory sex glands are inhibited by a product from the adrenal cortex.

SAMENVATTING

In dit proefschrift wordt een poging ondernomen om enkele effecten, die PRL heeft op reproductieve functies nader te bestuderen bij mannen met een PRL-producerend hypofyseadenoom.

Ter vergelijking wordt gebruik gemaakt van een proefdiermodel:

- Bij de rat wordt hyperprolactinaemie geinduceerd door subcutane inoculatie van een PRL- en ACTH-secernerende transplantabele hypofysetumor.
- De locale effecten van PRL op testiculaire functies worden bestudeerd door implantatie van een hypofyse in een testikel

Hyperprolactinaemie bij de man

De verschijnselen bij de man met hyperprolactinaemie als gevolg van een hypofyseadenoom worden, zoals uit vele studies (Hoofdstuk 1) blijkt, veelal bepaald door de invloed die het adenoom heeft op de omliggende structuren, gepaard gaande met hoofdpijnklachten, visusdaling en/of gezichtsvelduitval, oogspier parese en hypopituitarisme. Libidoverlies en impotentieklachten, welke retrospectief bij het merendeel van de mannen al geruime tijd aanwezig zijn, worden door vele auteurs als kenmerkend voor hyperprolactinaemie beschouwd.

Gynaecomastie en galactorrhoe zijn weinig frequent voorkomende verschijnselen.

Over het functioneren van het hypothalamus-hypofyse-gonadensysteem zijn de beschrijvingen nogal uiteenlopend. De basale waarden van LH en FSH kunnen nor-

maal of verlaagd zijn en vertonen een normale of verminderde response op toediening van het LRH. Het serum testosteron is vaak verlaagd, zelfs in de aanwezigheid van normale basale en met LRH gestimuleerde LH- en FSH-waarden. Bovendien kan het serum testosteron een volledig normale reactie vertonen na toediening van hCG. Een probleem bij het beoordelen van de vele onderzoekingen van hyperprolactinaemische mannen is, dat het merendeel van de mannen een macroadenoom heeft of dat er vaak al een behandeling is voorafgegaan aan het tijdstip van onderzoek.

In Hoofdstuk 3 worden de bevindingen bij 32 mannen met een PRL-producerend hypofyseadenoom besproken. Bij 19 van hen was er sprake van een macroadenoom. Zij presenteerden zich met klachten over hoofdpijn, gezichtsvelduitval en/of visusvermindering en hypopituitarisme. Het merendeel van deze 19 patiënten had daarnaast klachten over impotentie en libidoverlies. Bij lichamelijk onderzoek had 3 van de 19 mannen gynaecomastie en galactorrhoe. In deze groep van 19 mannen werden hoge serum PRL waarden gemeten. De basale waarden van LH en FSH waren normaal of laag normaal, doch de response op toediening van LRH was onvoldoende. Bovendien werden bij de meeste patiënten lage serum testosteronspiegels gemeten. Het was niet mogelijk om te onderscheiden of de gesupprimeerde hypofyse-gonadenas bij de 19 patiënten met een macroadenoom het gevolg was van de hoge serum PRL waarden, of dat de tumor zelf door compressie en/of destructie van het normale hypofyseweefsel aanleiding gaf tot functieverlies van de gonadotrope cellen. Deze laatste veronderstelling werd gesteund door het feit dat bij de 19 mannen met een macroadenoom ook andere hypofyse-voorkwabfuncties gestoord waren. Zowel uitval van de hypofyse-schildklieras, gemeten aan basale

en met TRH gestimuleerde TSH-concentraties, uitval van de hypofyse-bijnieras, gemeten aan 11-deoxycortisol waarden na toediening van metyrapone, als een volledig panhypopituitarisme werden gezien.

De overige 13 van de 32 hyperprolactinaemische mannen werden geselecteerd uit een groep van 598 mannen, die werden onderzocht wegens infertiliteit. Aangenomen werd dat de infertiliteit bij deze mannen berustte op de sperma afwijkingen. De lage incidentie van hyperprolactinaemie bij infertiliteit, zoals in de literatuur beschreven, werd bevestigd. Bij 11 van deze 13 patiënten waren er geen klachten over impotentie of libidoverlies, bij 2 van hen was er sprake van libidovermindering. De laatste 2 patiënten hadden een macroadenoom, de overigen hadden een microadenoom. Ten opzichte van de eerdergenoemde groep van 19 patiënten waren de PRL-spiegels veel lager. Gynaecomastie en galactorrhoe werd bij 2 van de 13 patiënten gezien.

In tegenstelling tot in de eerste groep waren de hypofysevoorkwabsfuncties en met name de basale en gestimuleerde LH- en FSH-waarden normaal. Bovendien waren de testosteronconcentraties veelal normaal. In deze groep van infertiele mannen met een microadenoom, waarbij locale compressie en/of destructie van het normale hypofyseweefsel waarschijnlijk niet voorkomt, is het weliswaar slechts licht verhoogde serum PRL niet in staat de hypofyse-gonadenas te onderdrukken.

Niettemin werd een behandeling met bromocriptine ingesteld om de effecten van normalisering van PRL op de fertiliteit te bestuderen. Twee mannen fertiliseerden nog voor de therapie, 4 mannen tijdens behandeling met bromocriptine. Bij deze 4 mannen was er geen verbetering van de spermakwaliteiten, wel zagen

wij een significante stijging van het serum testosteron, zoals ook in de literatuur beschreven wordt. Een andere in de literatuur veelvuldig vermelde bevinding was herstel van libido bij de 2 mannen met een macroadenoom in deze groep.

Op grond van deze gegevens wordt gesuggereerd dat bij patiënten met een PRL-producerend macroadenoom de suppressie van de hypofyse-gonadenas voornamelijk bepaald wordt door locale compressie en/of destructie van het normale hypofyseweefsel. Verbetering van libido en stijging van serum testosteronwaarden tijdens PRL verlagende therapie kunnen wijzen op een effect van PRL zelf.

Hyperprolactinaemie bij de rat

Naar aanleiding van mogelijke effecten van hyperprolactinaemie op de libido bij de man is het copulatiegedrag van de hyperprolactinaemische rat bestudeerd (Hoofdstuk 4). Hyperprolactinaemie werd geinduceerd door subcutane inoculatie met de PRL- en ACTH-secernerende tumor 7315a. Ongeveer 2 weken na inoculatie begon het serum PRL te stijgen om 5 tot 6 weken na inoculatie een concentratie van enkele microgrammen per ml te bereiken.

De aanwezigheid van de tumor leidde bij volwassen mannelijke ratten tot hypogonadotroop hypogonadisme (Hoofdstuk 5). Het feit dat de testosteronspiegel tijdens het groeien van de tumor sterk daalde maakte het noodzakelijk om het copulatiegedrag te bestuderen bij gegonadectomeerde dieren met constante serum testosteronwaarden door middel van subcutane plaatsing van een met testosteron gevuld silastic buisje. Tijdens het stijgen van de PRL concentratie werd een

opvallende verandering in het copulatiegedrag waargenomen. Er was een sterke toename van het aantal beklimmingen en het duurde langer, gerekend vanaf de eerste beklimming, tot de ejaculatie. Het aantal intromissies voorafgaande aan ejaculatie veranderde niet. De toename van het aantal beklimmingen was mogelijk het gevolg van een relatief onvermogen een goede intromissie te verkrijgen tijdens hyperprolactinaemie.

Aangezien de tumor zowel PRL als ACTH secerneert, hetgeen o.a. resulteert in een sterke vergroting van de bijnieren (Hoofdstuk 5) heben wij ook het copulatiegedrag van bijnierloze tumordragende dieren bestudeerd. Het copulatiegedrag van tumordragende bijnierloze dieren (die zeer hoge PRL-spiegels hadden) was identiek met dat van bijnierloze controledieren.

Op grond van deze waarneming kan worden geconcludeerd dat zelfs sterk uitgesproken hyperprolactinaemie geen invloed heeft op het copulatiegedrag van de bijnierloze mannelijke rat.

De in de literatuur vermelde resultaten van experimenten betreffende de invloed van PRL op LH, FSH en testosteron in het bloed bij de rat zijn nogal uiteenlopend (Hoofdstuk 2). Een matige PRL-verhoging, geinduceerd door ectopische hypofysen, gaat gepaard met een daling van de LH- en FSH-concentraties, terwijl het serum-testosteron normaal blijft. Ectopische hypofysen zijn tevens in staat om de stijging van serum LH en FSH, die optreedt na castratie, te onderdrukken. De inoculatie van PRL-secernerende tumoren bij de rat leidt tot sterk verhoogde PRL-spiegels, atrofie van testes en accessoire klieren en een verlaging van LH, FSH en testosteron.

In Hoofdstuk 5 worden de resultaten met betrekking tot LH, FSH, testosteron en gewichten van testes, ac-

cessoire klieren en bijnieren besproken bij volwassen mannelijke ratten met de PRL- en ACTH-secernerende tumor 7315a.

Tumorinoculatie bij intacte ratten leidde tot daling van LH, FSH en testosteron en tot een gewichtsdaling van testes en accessoire klieren, terwijl de bijnieren sterk in gewicht toenamen. Het mammakliergeweefsel was toegenomen en bevatte veel melk. De tumor was tevens in staat om de post-castratiestijging van LH en FSH te onderdrukken. Indien voor de tumorinoculatie adrenalectomie werd uitgevoerd vond er na castratie een normale stijging van het LH en FSH plaats, terwijl de LH- FSH- en testosteronspiegels bij uitsluitend geadrenalectomeerde tumordragende ratten niet veranderden ondanks de sterk stijgende PRL-concentraties in het serum. De waarnemingen bij gecastreerde ratten werden gedaan in de aanwezigheid van een constante, doch lage serum testosteronspiegel, verkregen door subcutane implantatie van een met testosteron gevuld silastic buisje. Bij autopsie bleek dat, ondanks het feit dat de serum testosteronwaarden bij geadrenalectomeerde en niet geadrenalectomeerde ratten ongeveer even hoog waren, er een daling optrad van de gewichten van accessoire klieren in de laatstgenoemde groep.

Vervolgens (Hoofdstuk 6) werd de regulatie van de LH- en FSH-secretie bij tumordragende dieren nader bestudeerd door bepaling van dopamine en LRH in hypofysesteelbloed. De afgifte van dopamine, de belangrijkste "PRL-inhibiting factor", wordt zoals uit vele studies blijkt via een short-loop positief feedback-mechanisme gereguleerd door PRL. Tevens is dopamine volgens sommige auteurs betrokken bij de secretie van LRH door de hypothalamus. Bij stoornissen in de secretie van LH en FSH tijdens hyperprolacti-

naemie zou dopamine een remmende factor kunnen zijn. De daling van LH en FSH in het serum bij intacte ratten na inoculatie van de 7315a tumor bleek verklaard te kunnen worden door een da- ling van LRH in het hypofysesteelbloed. Tevens waren zowel de dopamineconcentratie in het hypofysesteelbloed als de dopamineafgifte aan het hypofysesteelbloed verhoogd. Na adrenalectomie en tumorinoculatie werden gelijke LH- en FSH-concentraties gevonden in het serum van tumor- en niet-tumordragende ratten. De LRH-spiegels in het hypofysesteelbloed waren eveneens gelijk.
Daarentegen waren de dopaminewaarden in het hypofysesteelbloed van tumordragende dieren verhoogd.

De resultaten van experimenten beschreven in Hoofdstuk 5 en 6 wijzen erop dat hyperprolactinaemie bij bijnierloze dieren met de PRL- en ACTH-secernerende tumor 7315a niet leidt tot suppressie van serum LH en FSH. In Hoofdstuk 7 wordt beschreven, hoe is nagegaan welke factor uit de 7315a tumor, die naast PRL en ACTH tenminste ook nog β-endorphine secerneert, in staat is om in de aanwezigheid van de bijnieren de secretie van LH en FSH te onderdrukken. Subcutane toediening van ACTH aan volwassen, intacte mannelijke ratten veroorzaakte een daling van LH, FSH en testosteron in het serum en een vermindering van de gewichten van testes en accessoire klieren. Verder was de toediening van ACTH aan ratten, die gecastreerd werden, in staat om de te verwachten stijging van LH en FSH te onderdrukken. Bij gelijke testosteronwaarden, bij de gecastreerde ratten verkregen door de genoemde testosteron implantatie, waren de gewichten van de accessoire klieren van de met ACTH behandelde ratten ratten lager dan van die van de dieren, die geen ACTH kregen. Deze bevindingen zijn identiek met die bij dieren met de 7315a tumor zoals

beschreven in Hoofdstuk 5 en 6.

Ofschoon de hyperprolactinaemie bij bijnierloze dieren met de 7315a tumor geen invloed bleek te hebben op de testosteronconcentratie in het serum (Hoofdstuk 5) worden in de literatuur (Hoofdstuk 2) aan PRL ook stimulerende effecten op de functie van de gonaden van de rat toegeschreven.

In Hoofdstuk 8 wordt nader onderzoek van de gonadale functies beschreven. Door implantatie van een hypofyse in 1 testikel werden zowel locaal in het weefsel als in het testiculaire veneuze bloed van de testikel met een hypofyse transplantaat hoge PRL-waarden verkregen, terwijl in het perifere veneuze bloed geen hyperprolactinaemie ontstond. 100 Dagen na de implantatie was het testosterongehalte van de gehele testikel met de ectopische hypofyse hoger dan van de andere testikel. Noch in het testiculaire veneuze bloed, noch in het perifere veneuze bloed werden veranderingen van de testosteronconcentraties gezien. Verder bleek de spermatogenese, zelfs in tubuli direct naast het hypofysetransplantaat gelegen, volledig ongestoord te zijn.

De in dit proefschrift beschreven experimenten geven geen steun aan de in de literatuur geldende opvatting dat PRL zelf bij de man en de mannelijke rat aanleiding geeft tot stoornissen in de voortplanting. In de mannelijke rat met de PRL- en ACTH-secernerende 7315a tumor is de aanwezigheid van de bijnier vereist voor de daling van LH, FSH en testosteron.
De door farmacologische doses ACTH gestimuleerde bijnier blijkt in staat te zijn de LH-, FSH- en testosteronsecretie te onderdrukken. Ook de stimulerende werking van testosteron op accessoire klieren wordt geremd door een bijnierschorsproduct.

NAWOORD

Het schrijven van een wetenschappelijk verslag is slechts de summiere weergave van een onderzoek waaraan de schrijver tegenwoordig vaak een bescheiden aandeel heeft geleverd. Velen ben ik dan ook dank verschuldigd bij het volbrengen van dit proefschrift. Mijn dank gaat in de eerste plaats uit naar de afdelingen Biochemie II (Hoofd, Prof. Dr. H.J. van der Molen), Fysiologie II (Hoofd, Prof. Dr. J.J. van der Werff ten Bosch) en Inwendige Geneeskunde III (Hoofd, Prof. Dr. J.C. Birkenhäger), die mij in staat hebben gesteld om in 1979 een aanvang te maken met het onderzoek, dat voor een groot deel plaats vond op de afdeling Fysiologie II. Met veel plezier denk ik terug aan de ongedwongen werksfeer op deze afdeling, waar Jan Vreeburg mij zeer vele aspecten van het bedrijven van wetenschap heeft bijgebracht en ik mag mij gelukkig prijzen dat de hechte samenwerking die hieruit ontstaan is de komende jaren voortgezet kan worden. Veel dank ben ik ook verschuldigd aan Steven Lamberts, die als mede-projectleider met enthousiasme en opbouwende kritiek mij heeft gestimuleerd de vele ideeën, die tijdens het onderzoek ontstonden, verder uit te werken. Niet weg te denken in dit samenwerkingsverband is Marja Ooms, die het merendeel van de laboratoriumbepalingen heeft gedaan en voortdurend bereid was en is om behulpzaam te zijn bij de vele praktische aspecten van experimenteel werk. Frank de Jong en Focko Rommerts maakten mij vertrouwd met de isolatie-technieken van Leydigcellen en de sampling van testiculair veneus bloed. De vaak heftige discussies met hen zijn onvergetelijk. De mogelijkheid om op 3 afdelingen werkzaam te zijn biedt uiteraard

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 28 mei 1946 te Rotterdam geboren. In 1964 behaalde hij het diploma HBS-B aan het Charloise Lyceum te Rotterdam. Op 12 februari 1971 legde hij het artsexamen aan de Rijks Universiteit te Leiden af. Van 1971 tot 1973 vervulde hij zijn militaire dienstplicht bij de Koninklijke Marine. In deze periode was hij 16 maanden werkzaam op de interne afdeling van het Marine Hospitaal te Overveen (A.D.J. Verburg). Na gedurende 9 maanden in opleiding te zijn geweest op de kinderafdeling van het Elisabeth Ziekenhuis te Tilburg (Dr. D.J. van Zaane) werd in october 1973 een aanvang gemaakt met de opleiding tot internist (Opleider: Dr. P.S. Blom). In 1978 volgde inschrijving in het specialistenregister. Sindsdien is hij werkzaam op de afdeling Inwendige Geneeskunde III van het Academisch Ziekenhuis te Rotterdam (Hoofd: Prof. Dr. J.C. Birkenhäger).